91-02381/

SCIENTIFIC REPORT 1989/1990

MANITOBA CANCER TREATMENT
AND RESEARCH FOUNDATION

INTRODUCTION

The Honourable Donald W. Orchard,
Minister of Health

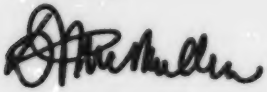
Dear Mr. Orchard:

I have the honour of forwarding the following report on behalf of the Manitoba Cancer Treatment and Research Foundation. This year marks the beginning of the planning process for an expanded cancer treatment facility at its Olivia Street location on the Health Sciences Centre campus. The need for such an expansion of both patient care and research facilities has been dictated, not only by the increasing number of patients which the centre has seen and treated, but the increasing sophistication of such treatment which has now expanded beyond the capacity of the Foundation. This building program will be made possible by a joint effort of public subscription and government funding. The public response to the need has been most encouraging and is an indication both of the need and of the support which the Foundation has amongst Manitobans.

The year has been marked by some major gains in modalities of cancer care including the beginning of installation of a 20 MEV linear accelerator and a smaller 6 MEV unit. This modernization as well as updating of treatment facilities is an essential component of maintaining state of the art therapy for our patients. In addition to this, it has been a year of major advances within the cancer research segment of the Foundation, including the continuing development of a cancer treatment advisory system which will place computer technology and computer generated advice in the Outreach centres of Manitoba.

At this time I would like to express my personal thanks to the staff of the Foundation whose dedication to patient care and to pushing back the frontiers of cancer medicine is exemplary.

Yours truly,



D. J. McMullin, C.A.
Chairman

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THE MANITOBA CANCER TREATMENT AND RESEARCH FOUNDATION

Board of Directors

D. J. McMullin, *Chairman*
B. Adams
J. Anderson
I. Auld (effective October 1989)
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A. Bishop, M.D.
L. Crane
L. Duncan
F. Edwards (to April 1989)
N. Hilderman (effective October 1989)
G. Horne
A. M. Lysack, M.D.
J. Rodie

Ex Officio
The Honourable D. W. Orchard, *Minister of Health*
J. Foerster, M.D., *Chairman, Advisory Medical Board*

Executive Finance Committee

G. Horne, *Chairman*
B. Adams
L. Crane
D. McMullin

Advisory Medical Board

J. Foerster, M.D., B.Sc., (Med.), *Chairman*
E. Baral, M.D., Ph.D.
W. Beecroft, M.D., D.D.S. (Tor.), F.R.C.S. (C)
D. Bowman, M.D., B.Sc. (Med), F.R.C.P.(C)
R. L. Cooke, B.Sc., M.D., F.R.C.S. (C)
A. L. Davidson, M.D., M.B., Ch.B., F.R.C.S. (C)
M. H. Day, B.Sc. (P.E.), M.D., C.C.F.P.
P. A. Galbraith, M.Sc., M.D., F.R.C.P. (C), F.A.C.P.
G. V. Krepart, B.Sc., M.D., F.R.C.S. (C), B.C.A.M. (Ob/Gyn), F.G.O., S.B.C.G.O.
L. G. Israels, B.A., M.D., M.Sc., F.R.C.P. (C)
I. C. Peever, M.D., F.A.C.O.G., F.S.O.G.O., F.R.C.S. (C)
B. Weinerman, M.D., F.R.C.P. (C)

Scientific Advisory Committee

H. Duckworth, B.Sc., Ph.D.
Professor, Dept. of Chemistry
University of Manitoba

A. Froese, B.Sc., Ph.D.
Professor, Dept. of Immunology
University of Manitoba

F. Labella, B.A., M.A., Ph.D.
Professor, Dept. of Pharmacology and Therapeutics
University of Manitoba

Ex Officio

L. G. Israels, M.D.
Executive Director
Manitoba Cancer Treatment & Research Foundation

MANITOBA INSTITUTE OF CELL BIOLOGY

Board of Management

R. Andison, *Chairman* (to February 1989)
D. J. McMullin, *Chairman* (effective February 1989)
N. Anthonisen, M.D.
S. Berney
R. Best
A. Bishop, M.D.
M. Vaisey-Genser
A. Greenberg, M.D., Ph.D. — *Ex Officio*
Mr. Justice G. Hall
L. G. Israels, M.D.
J. Jamieson, Ph.D.

Administration

Executive Director — L. G. Israels, M.D.
Assistant Executive Director — R. R. Publow
Executive Secretary — J. Morton
Administrative Assistant Finance — I. Birse
Administrative Assistant Personnel — D. Lamontagne
Accounts Payable — M. Gemmell

Professional Staff

The Foundation is an integral part of the teaching and research program of the University of Manitoba. Various staff members participate in the undergraduate oncology program, teach on the hospital wards, and have graduate students in their laboratories.

UNIVERSITY APPOINTMENTS

Faculty of Medicine

Department of Anatomy
Dr. G. Froese, *Associate Professor*

Department of Biochemistry
Dr. J. Wright, *Professor*
Dr. L. G. Israels, *Adjunct Professor*

Department of Human Genetics
Dr. A. H. Greenberg, *Professor*

Department of Immunology
Dr. E. Paraskevas, *Professor*
Dr. A. Greenberg, *Professor*
Dr. D. A. Chow, *Assistant Professor*

Department of Internal Medicine
Dr. L. G. Israels, *Distinguished Professor*
Dr. E. Baral, *Professor*
Dr. D. Bowman, *Professor*
Dr. G. J. Goldenberg, *Professor*
Dr. F. Paraskevas, *Professor*
Dr. B. Schacter, *Professor*
Dr. B. Weinerman, *Professor*
Dr. L. Brandes, *Associate Professor*
Dr. M. Levitt, *Associate Professor*
Dr. H. Schipper, *Associate Professor*
Dr. A. Begleiter, *Assistant Professor*
Dr. E. Bow, *Assistant Professor*
Dr. J. Johnston, *Assistant Professor*
Dr. H. Y. P. Lam, *Assistant Professor*
Dr. T. Shore, *Assistant Professor*
Dr. J. Wright, *Adjunct Professor*

Department of Obstetrics and Gynaecology
Dr. V. G. Krepart, *Professor*
Dr. R. J. Lotocki, *Assistant Professor*

Department of Pediatrics
Dr. A. Bishop, *Professor*
Dr. A. H. Greenberg, *Professor*
Dr. J. M. Gerrard, *Associate Professor*
Dr. E. D. Israels, *Associate Professor*
Dr. M. Schroeder, *Associate Professor*
Dr. S. J. Israels, *Assistant Professor*
Dr. N. Kobrinsky, *Assistant Professor*

Department of Physiology
Dr. J. Dodd, *Assistant Professor*
Dr. M. Mowat, *Assistant Professor*
Dr. A. H. Greenberg, *Adjunct Professor*

Department of Radiology
Dr. E. Baral, *Professor*
Dr. K. Malaker, *Associate Professor*
Dr. S. Shalev, *Associate Professor*
Dr. P. Dunscombe, *Assistant Professor*
Dr. G. Froese, *Assistant Professor*
Dr. P. Hahn, *Assistant Professor*
Dr. W. Huda, *Assistant Professor*
Dr. K. D. Jones, *Assistant Professor*
Dr. W. McLellan, *Assistant Professor*
Dr. R. E. Palser, *Assistant Professor*
Dr. S. E. Sayed, *Assistant Professor*
Dr. A. Sourkes, *Assistant Professor*
Dr. E. Hadzic, *Lecturer*
Dr. S. Larsson, *Lecturer*
Dr. K. Vijayaraghavan, *Lecturer*

Department of Surgery
Dr. E. Ramsey, *Professor*
Dr. B. J. Anderson, *Assistant Professor*
Dr. W. Beecroft, *Associate Professor*

Department of Social and Preventive Medicine
Dr. N. W. Choi, *Professor*
Dr. N. A. Nelson, *Associate Professor*
Mrs. P. F. Moodie, *Lecturer*

Faculty of Science

Department of Microbiology
Dr. J. Wright, *Professor*

Department of Physics
Dr. P. Dunscombe, *Adjunct Professor*
Dr. G. Froese, *Adjunct Professor*
Dr. W. Huda, *Adjunct Professor*
Dr. R. Palser, *Adjunct Professor*
Dr. G. Sandison, *Adjunct Professor*
Dr. S. Shalev, *Adjunct Professor*

Computer Science
Dr. D. Scuse

Department Heads

Clinical Programs
Dr. Brent Schacter, M.D., FRCPC

Epidemiology and Biostatistics
Dr. N. W. Choi, M.D., M.P.H., Ph.D., FACE

Gynecological Oncology
Dr. Garry Krepart, M.D., B.Sc.(Med), FRCS(C), FACOG

Hematology Laboratory
Mrs. Susan Tighe, R.T.

Information Services
Dr. Harvey Schipper, B.A.Sc., M.D., FRCPC
Mr. Peter West

Library
Ms. Donna Chornenki

Medical Oncology
Dr. David M. Bowman, M.D., B.Sc.(Med), FRCPC

Medical Physics
Dr. Peter Dunscombe. Ph.D., FCCPM

Nursing and Technology
Ms. Beverley Glenday, R.N.

Pediatric Hematology/Oncology
Dr. Nathan Kobrinsky, B.Sc.(Med), M.D., FAAP, FRCPC
Dr. Jon Gerrard, M.M., Ph.D., FRCPC

Pharmacy
Ms. Diana Dickof, B.Sc., B.Sc.Pharm.

Psychiatric Oncology Service
Dr. Harvey Chochinov, M.D., FRCPC

Radiation Oncology
Dr. Edward Baral, M.D., Ph.D.

Radiotherapy Treatment Floor
Ms. Joan Howes, R.T.T.

Records and Registry
Dr. Harvey Schipper, B.A.Sc., M.D., FRCPC
Ms. Gloria Hanson, CCHRA(A)

Social Work
Mrs. Betty Wong, M.S.W., R.S.W.

Surgical Oncology
Dr. Wayne Beecroft, D.D.S., M.D., FRCPC, FACS

Urologic Oncology
Dr. Eric Ramsay, M.B., BCh., FRCPC

Volunteer Services
Mrs. Elaine Hickerson, B.A.

St. Boniface General Hospital

Oncology Unit
Dr. Harvey Schipper, B.A.Sc., M.D., FRCPC

Programmes

Cancer Outreach Programme
Dr. Harvey Schipper, B.A.Sc., M.D., FRCPC
Mrs. Anne Nemecek, R.N., P.H.N.

Canadian Cancer Society Volunteer Services
Mrs. Helen Morrow

RESEARCH REPORTS

MANITOBA INSTITUTE
OF CELL BIOLOGY

I. ORGANIZATION

ADMINISTRATION

Arnold H. Greenberg, M.D., Ph.D., *Director*
Jim A. Wright, Ph.D., *Associate Director*
Nikki Ryan, *Administrative Assistant*

BOARD OF MANAGEMENT

Dennis J. McMullin, *Chairman*
Richard Addison, *Member-at-Large*
Nick Anthonisen, *Dean, Faculty of Medicine*
Sheldon Berney, *Member-at-Large*
Bob Best, *Canadian Cancer Society*
Agnes Bishop, *Head, Department of Pediatrics, University of Manitoba*
Justice Gordon Hall, *Member-at-Large*
Lyonel Israels, *Executive Director, Manitoba Cancer Foundation*
James Jamieson, *Head, Department of Chemistry, University of Manitoba*
Marion Vaisey-Genser, *Vice-President (Research), University of Manitoba*

SCIENTIFIC STAFF:

SENIOR MEMBERS:

Asher Begleiter, Ph.D.
Donna Chow, Ph.D.
Gerald J. Goldenberg, M.D., Ph.D.
Sara J. Israels, M.D.
H.Y. Peter Lam, Ph.D.
Frixos Paraskevas, M.D.
Jim Wright, Ph.D.
Lorne J. Brandes, M.D.
Jon M. Gerrard, M.D., Ph.D.
Arnold H. Greenberg, M.D., Ph.D.
James Johnston, M.D.
Michael Mowat, Ph.D.
Brent A. Schacter, Ph.D.

ASSOCIATE MEMBERS:

Janice Dodd, Ph.D.
E.D. Israels, Ph.D.

RESEARCH ASSOCIATES AND POSTDOCTORAL FELLOWS:

Mohamed Abdelhaleem, M.D.
Mohammed Deffie, Ph.D.
Robert Hurta, Ph.D.
Archie McNicol, Ph.D.
Satya Saxena, Ph.D.
Lianfa Shi, Ph.D.
Richard Brown, Ph.D.
Lori Ebbeling, M.D.
Nidhi Jain, Ph.D.
Shanti Samuel, Ph.D.
Lois Schwarz, Ph.D.
Maureen Spearman, Ph.D.

GRADUATE STUDENTS:

Asim Ashique, B.Sc.
Racquel Baert, B.Sc.
Christine Chiu, B.Sc.
Jacqueline Damen, B.Sc.
Jason Falk, B.Sc.
Stephen Hrushovetz, B.Sc.
Eugene Roussel, M.Sc.
Susan Battistuzzi, B.Sc.
Yongzhong Chen, B.Sc.
Bob Choy, B.Sc.
Sean Egan, B.Sc.
Geoffrey Hicks, B.Sc.
Ricky Kraut, B.Sc.
Paul Sandstrom, B.Sc.

Gerald Stelmack, B.Sc.
David Tough, B.Sc.
Catherine Vriend, B.Sc.
Wenzheng Yang, B.Sc.
Esther Taylor, B.Sc.
Shashi Uniyal, B.Sc.
Ping Yang, M.Sc.
Li Zuo, M.D.

SUMMER STUDENTS:

Racquel Baert
Mitch Bruneau
Lorne Janz
Raminder Nirula
Anne Robbins
Bruce Ballon
Gerald Eschun
Joanna Lynch
Martin Reese
Paul Syslak

TECHNICIANS:

Aaron Abrams
Oliver Bereznay
Dan Bosman
Arthur Chan, M.Sc.
Michele Daniels
John Dickson
Pauline Gerrard
Shelly James
Michael Jesson
Katayun Kamali
Marsha Leith
Eileen McMillan
Catherine Robertson
Erica Robotham
Rupinder Singal
Balram Sukhu
Michael Talgoy
Linda Verburg
Cynthia Anhalt
Pat Bogdanovic
Alice Chan
Valerie Cripps
Lynn Desilets
Elena Duta
Nina Hunt
Lenka Jarolim
Henry Joling
Angela Kemp
Donald Lint
Sandra Oh
Pauline Robinson
Mary Sauder
Sandra Singhroy
Anand Sukhan
Kristine Tang

LAB ASSISTANTS:

Irene Freiter
Maria Monch
Adi Limpach
Inga Tober

SECRETARIES:

Marisa Kemper
Nikki Ryan
Agnes Warkentin
Glenys Morgan
Susan Snusher

VISITING SCIENTISTS:

Dr. Svetlana Dolina, *Ben Gurion University, Israel*
Dr. Lev Hatzkellson, *Ben Gurion University, Israel*
Dr. Hans Lutz, *Swiss Federal Institute of Technology, Zurich*

THE MANITOBA INSTITUTE OF CELL BIOLOGY

INTRODUCTION

The Manitoba Institute of Cell Biology was founded jointly by the University of Manitoba and the Manitoba Cancer Treatment and Research Foundation in 1969. The purpose of the Institute is to carry out research and training in cancer and other diseases by studying disease mechanisms at a cellular and molecular level. The Institute of Cell Biology is governed by an independent Board of Management consisting of eleven members, three appointed by the University of Manitoba, three appointed by the Manitoba Cancer Treatment and Research Foundation and five jointly appointed by both sponsoring Institutions. The Manitoba Institute of Cell Biology is housed in the Manitoba Cancer Treatment and Research Foundation adjacent to the Faculty of Medicine, University of Manitoba and the Health Sciences Centre.

The Institute of Cell Biology consists of a Director, twelve senior investigators, and ninety-five staff including research associates, postdoctoral fellows, graduate students, technicians, laboratory assistants and secretaries. Although the Institute of Cell Biology is not a degree-granting body, advanced degrees at the Masters or Ph.D. level may be obtained with any of the departments in the Faculty of Medicine, University of Manitoba. The major emphasis of the research program of the Institute of Cell Biology has centered on the fields of Molecular Biology of Tumor Progression, Biochemical Pharmacology of Drug Resistance, Immunology and Hematology. Senior investigators hold cross-apppointments in many University departments including the Departments of Immunology, Genetics, Biochemistry, Physiology, Microbiology, Medicine and Pediatrics.

The location of the Manitoba Institute of Cell Biology in the Manitoba Cancer Treatment and Research Foundation building has enabled the development of parallel programs of basic and clinical investigation of patients with neoplastic disease. Patients with a wide range of neoplastic disorders are available for investigation conducted by senior investigators in the Institute. Not only is this interaction facilitated by a geographical juxtaposition of the two Institutions, but also by the fact that many senior investigators hold cross-appointments in the Manitoba Institute of Cell Biology and Manitoba Cancer Treatment and Research Foundation.

The Manitoba Institute of Cell Biology is only one of two institutes in Canada funded by the National Cancer Institute, and has been continually supported by the NCI since the inception of the Institute group in 1969. During the 1988-89 academic year, members of the Institute raised $3.02 million dollars in research funds, of which $0.7 million was for career awards, postdoctoral fellowships and studentships. Funding sources include the NCIC, MRC, NSERC, NIH, many smaller foundations, as well as private industry (Bristol-Myers, Park Davis, RJ Reynolds). This represents one of the highest per capita funding rates of all university Departments.

Dr. Arnold H. Greenberg

REPORT OF THE DIRECTOR

ARNOLD H. GREENBERG, M.D. Ph.D.

Over the 21 years of the Institute's history the emphasis of our approach to cancer research has changed, with greater effort being directed towards elucidating mechanism of disease at the cellular and molecular level, and, as a consequence, much more of this research is being done in basic science laboratories. The shift from clinical to basic research in our Institute has been part of a trend in all biomedical research institutions during this time period. This was, in part, a reflection of the policy of funding agencies such as the N.C.I.C. to develop basic molecular research in Canada. Because of this, many research institutes now have researchers who are focused on basic biological problems which have no obvious relevance to the disease process. It seems to us, that a balance must be found in research groups between the scientists needed for successful basic research which underpins most of our scientific advances, and the 'interface' scientists with medical training who can speed the application of these ideas to medical problems. If we are to succeed in convincing governments at the Federal and Provincial level to continue supporting our biomedical research institutes, we must make sure that basic research discoveries are exploited fully, and that the government and the public are made aware of the advances being made and their benefit to society. This is particularly true for our Institute, which is not directly funded by the University and relies primarily upon the generosity of the public.

Our Institute is fortunate enough to have a great deal of support from the Manitoba Cancer Research and Treatment Foundation through their continuing funding of our laboratories and core facilities. The Foundation is also looking to the future in its efforts to expand research space and research staff with a proposal for the new building which will include a much larger Institute. As a part of the planning process, the Institute convened a Retreat during the summer of 1989 to discuss priority areas for development. On the basis of this meeting, we have begun recruiting several new investigators, using funding both from the University of Manitoba Faculty Development Fund and the Manitoba Cancer Treatment and Research Foundation Core Budget to the Institute. It is anticipated that three new investigators will be hired prior to the building expansion in 1994, and this will be followed by a second round of recruitment when the new research space is available. Initial recruitment efforts will be directed at enriching our molecular oncology program, as well as developing a facility for protein sequencing and a murine transgenic unit. As part of our recruitment efforts, we are pleased to announce the appointment of Dr. Eva Turley. Dr. Turley obtained her Ph.D. at U.B.C. and her post-doctoral training at Johns Hopkins University and the University of Oregon. She will be moving from the University of Calgary where she is now an Associate Professor and Head of the Metastasis Research Unit, in September of 1990. Dr. Turley's research program is on the molecular mechanisms of cell locomotion, and most recently on the role of hyaluronate and the hyaluronate receptor in signalling for initiation of cell motility. Her research has received international attention and is acknowledged by colleagues in the field as a highly original and exciting area of research. Dr. Turley will be joining the molecular oncology group and has already initiated collaborations with other scientists in our Institute who have interests in tumor cell invasion and metastasis.

AWARDS AND HONOURS

DONNA A. CHOW, Ph.D.

was a SYMPOSIUM SPEAKER at the Society for *Biological Therapy*, Immunity to Cancer, Williamsberg, Virginia.

JON GERRARD, M.D., Ph.D.

was awarded the GREENBERG SCHOLARSHIP from the *Oklahoma Medical Research Foundation*, Oklahoma City, OK.

ARNOLD H. GREENBERG, M.D., Ph.D.

was invited to be a KEYNOTE SPEAKER at the *Yardley Symposium on Neuroimmunology*, John's Hopkins Hospital, Baltimore, Maryland.

was appointed ASSOCIATE EDITOR, *Cancer Metastasis Revieus*.

was appointed CHAIRMAN, *Terry Fox Program Project Panel* of the National Cancer Institute of Canada.

has received a *National Cancer Institute* TERRY FOX CANCER RESEARCH SCIENTIST AWARD.

JIM WRIGHT, Ph.D.

was a SYMPOSIUM SPEAKER at the *Fourth Rossiter Research Conference* on Genetics and Disease, Lake Couchiching, Ontario.

was an INVITED SPEAKER at the *Ribonucleotide Reductase Conference* in Hemavan, Sweden.

was an INVITED PARTICIPANT at the *Keble Conference* in Oxford, England.

was awarded the Robert A. Macbeth Travelling Fellowship of the Canadian Cancer Society, Manitoba Division

became a TERRY FOX CANCER RESEARCH SCIENTIST AWARDEE of the *National Cancer Institute of Canada*.

POST-DOCTORAL AND STUDENT AWARDS:

MOHAMMED ABDELHALEEM, M.D.

received a STEVE FONYO STUDENTSHIP of the *National Cancer Institute of Canada*.

BOB CHOY, B.Sc.

received a *Manitoba Health Research Council* STUDENTSHIP and has been awarded a *National Career Institute of Canada* POSTDOCTORAL FELLOWSHIP.

JAQUELINE DAMEN, B.Sc.

received a *University of Manitoba* GRADUATE STUDENT FELLOWSHIP.

SEAN EGAN, Ph.D.

won the PROWSE PRIZE for the outstanding Thesis presentation in the *Faculty of Medicine*.

ROBERT HURTA, Ph.D.

received the *GEORGE SELLERS* POSTDOCTORAL FELLOWSHIP.

NIDHI JAIN, Ph.D.

was awarded a Post-Doctoral FELLOWSHIP from the *University of Manitoba Faculty Fund.*

LORNE JANZ, B.A.

received a *National Science and Engineering Research Council* STUDENTSHIP.

NASREEN KHALIL, M.D.

received a *PARKER FRANCIS FOUNDATION* SCHOLARSHIP.

SATYA SAXENA, Ph.D.

received a *Manitoba Health Research Council Post-Doctoral* FELLOWSHIP

LOIS SCHWARZ, Ph.D.

received a *Manitoba Health Research Council Postdoctoral* FELLOWSHIP.

MAUREEN SPEARMAN, Ph.D.

received a *Medical Research Council Postdoctoral* FELLOWSHIP.

WILLIAM TAYLOR, B.Sc.

received a *National Science and Engineering Research Council* STUDENTSHIP and a *University of Manitoba* GRADUATE STUDENT FELLOWSHIP.

DAVE TOUGH, B.Sc.

received a *University of Manitoba Alumni Association Graduate* FELLOWSHIP

won the MERCK FROSST CANADA AWARD in Cell Biology for the Thesis competition in the *Faculty of Medicine.*

won the CLARENCE BOGARDUS SHARPE MEMORIAL PRIZE for the student ranking highest in the *University of Manitoba Fellowship competition.*

WENZHENG YANG, B.Sc.

received a *Manitoba Health Research Council* STUDENTSHIP

VISITING SEMINAR SPEAKERS

The Role of Cathepsin D in the Release of Golgi Bound Sialyltransferase During the Acute Phase Response
Jim Jamieson, Ph.D., *Department of Chemistry, University of Manitoba*

Molecular Characterization of Prostate Disease
Jan Dodd, Ph.D., *Department of Physiology, University of Manitoba*

Progesterone Derivatives as Putative Endogenous Regulators of Sodium, Potassium-ATPase
Frank LaBella, Ph.D., *Department of Pharmacology, University of Manitoba*

Tuftsin, an Immunomodulator and Potential Antitumor Agent
R. Catane, M.D., *Associate Professor, Department of Radiation and Clinical Oncology, Hadassah Medical Organization, Jerusalem, Israel*

Citrate Synthase: Site Directed Mutagenesis of an Allosteric Enzyme
Harry W. Duckworth, Ph.D., *Department of Chemistry, University of Manitoba*

Physiological and Pharmacological Modulation of Cardiac Contractivity
Depak Bose, Ph.D., *Department of Pharmacology, University of Manitoba*

Characterization of Human Immune Response to Murine Antibodies by Solid Phase ELISA
David Beatty, M.D., *Division of Surgery, City of Hope National Medical Center, Duarte, California*

Expression and Function of the K-fgf Oncogene
Claudio Basilico, Ph.D., *New York University, New York, New York*

Insulin-like Growth Factor-1
Liam J. Murphy, M.D., Ph.D., *Department of Physiology, University of Manitoba*

Interactions Between the Nervous and Immune Systems
David Felten, Ph.D., *University of Rochester, Rochester, New York*

The Role of Glutathione S-Transferase in Resistance
Kenneth Tew, Ph.D., *Fox Chase Cancer Center, Philadelphia, Pennsylvania*

The Role of TNF-∝ in Macrophage Activation
Neidi J.-H. Gong, Ph.D., *Institute of Immunology, University of Marburg, West Germany*

Thrombospondin: Structure and Function and Role in Tumor Metastasis
Valerie Castle, M.D., *Mott Children's Hospital, University of Michigan, Ann Arbor, Michigan*

Gene Regulation by Growth Factors and Oncogenes
Edward Ziff, Ph.D., *Department of Biochemistry, New York University, Medical Center, New York, New York*

The Role of ras GTPase Activating Protein (GAP) in Signal Transduction
Frank McCormick, Ph.D., *Cetus Corp., Emeryville, California*

Structure, Function and Expression of the c-rel Proto-Oncogene
Mark Hannink, Ph.D., *McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin*

Functional Consequences of Platelet Derived Growth Factor Receptor Phosphorylation
Andrius Kazlauskas, Ph.D., *Fred Hutchinson Cancer Center, Seattle, Washington*

Lymphocytic Adhesion: Structural and Functional Studies
John Wilkins, Ph.D., *Rheumatic Disease Unit, University of Manitoba*

Molecular Analysis of Chromosomal Abnormalities in T Cell Leukemias
Lawrence Finger, Ph.D., *Fels Institute for Cancer, Temple University, Philadelphia, Pennsylvania*

Phosphorylation Mediated Signalling Between Tyrosine Kinases and p21ras-Associated Proteins
Michael Moran, Ph.D., *Mount Sinai Hospital Research Institute, Toronto, Ontario*

The Retinoblastoma Protein in Transformation and Differentiation
Peter Whyte, Ph.D., *Fred Hutchinson Cancer Research Center, Seattle, Washington*

II. MOLECULAR ONCOLOGY

SENIOR MEMBERS

Arnold H. Greenberg, M.D., Ph.D.
Michael Mowat, Ph.D.
Jim Wright, Ph.D.

RESEARCH ASSOCIATES AND POSTDOCTORAL FELLOWS:

Robert Hurta, Ph.D.
Lois Schwarz, Ph.D.
Maureen Spearman, Ph.D.
Shanti Samuel, Ph.D.
Lianfa Shi, Ph.D.

GRADUATE STUDENTS:

Asim Ashique, B.Sc.
Christine Chiu, B.Sc.
Jacqueline Damen, B.Sc.
Geoffrey Hicks, B.Sc.
Ping Yang, M.Sc.
Racquel Baert, B.Sc.
Bob Choy, B.Sc.
Sean Egan, B.Sc.
Ricky Kraut, B.Sc.
Wenzheng Yang, B.Sc.

SUMMER STUDENTS:

Racquel Baert
Anne Robbins
Bruce Ballon
Paul Syslak

TECHNICIANS:

Aaron Abrams
John Dickson
Mary Sauder
Arthur Chan, M.Sc.
Angela Kemp, M.Sc.
Michael Talgoy, M.Sc.

Dr. Jim A. Wright

THE ACTIVATED FORM OF P53 IS NOT A TRANSACTIVATOR OF THE INTRACISTERNAL A PARTICLE LONG TERMINAL REPEAT PROMOTER

M. Mouvat, M. Sauder, D. Pereira

Published observations have suggested that the activated form of p53 protein could transactivate the Intracisternal A particle long terminal repeat promotor (IAP-LTR). In this paper we demonstrate that the increased expression from this promoter was due to effects of the co-transferred plasmids and not p53 per se. In transient expression experiments, co-transfer of either a p53 or a p53 frame-shift mutant plasmid with an IAT-LTR driven chloramphenicol acetyl transferase (CAT) plasmid gave a similar increase in CAT activity. Further, this increase in CAT gene activity could also be achieved by co-transfer of a plasmid containing a viral promoter alone.

EXPRESSION OF WILD TYPE P53 IS NOT COMPATIBLE WITH CONTINUED GROWTH OF P53-NEGATIVE TUMOR CELLS

P. Johnson, D. Gray, M. Mouvat, S. Benchimol

Inactivation of the cellular p53 gene is a common feature of Friend virus-induced murine erythroleukemia cell lines and may represent a necessary step in the progression of this disease. As well, frequent loss or mutation of p53 alleles in diverse human tumours is consistent with the view of p53 as a tumour suppressor gene. To examine the significance of p53 inactivation in tumorigenesis, we have attempted to express transfected wild type p53 in three p53-negative tumour cell lines: murine DP16-1 Friend erythroleukemia cells, human K562 cells and SKOV-3 cells. Here we show that aberrant p53 proteins, which differ from wild type p53 by a single amino acid substitution, are expressed in these cells, whereas wild type p53 expression is not tolerated. The inability of p53-negative tumour cell lines to support wild type p53 expression is consistent with the view of p53 as a tumour suppressor.

TRANSIENT ELEVATION OF RIBONUCLEOTIDE REDUCTASE ACTIVITY, M2 mRNA AND M2 PROTEIN IN BALB/c 3T3 FIBROBLASTS IN THE PRESENCE OF 12-0-TETRADECANOYL-PHORBOL-ACETATE

B.K. Choy, G.A. McClarty and J.A. Wright

A rapid elevation of ribonucleotide reductase activity was observed with BALB/c 3T3 fibroblasts within 1/2 to 1 hour treatment with 0.1 μM 12-0-tetradecanoylphorbol-13-acetate (TPA). This increase in activity was transient, and returned to about normal levels within 24 to 28 hours. Northern analysis of the two components of ribonucleotide reductase showed a slight transient elevation of M1 mRNA and a marked transient elevation of M2 mRNA after 1/2 hour TPA treatment. As a positive control, ornithine decarboxylase message levels were also observed to be transiently elevated following identical treatment with TPA. Western blot analysis with M1 and M2 specific monoclonal antibodies indicated that the increase in elevation of the M2 but not the M1 protein during treatment with 0.1 μM TPA. This first demonstration that the tumor promotor, TPA, can cause rapid and transient alterations in ribonucleotide reductase suggests that the enzyme, particularly the M2 component, may play an important role in the critical early events involved in the process of tumor promotion.

RELATIONSHIPS BETWEEN SENSITIVITY TO HYDROXYUREA AND 4-METHYL-5-AMINO-1-FORMYLISOQUINOLINE THIOSEMICARBAZONE (MAIQ) AND RIBONUCLEOTIDE REDUCTASE RNR2 mRNA LEVELS IN STRAINS OF *SACCHAROMYCES CEREVISIAE*

D.A.H. Rittenberg and J.A. Wright

Ribonucleotide reductase is responsible for providing the deoxyribonucleotide precursors for DNA synthesis. In most species the enzyme consists of a large and a small subunit, both of which are required for activity. In mammalian cells, the small subunit is the site of action of several antitumor agents, including hydroxyurea and 4-methyl-5-amino-1-formylisoquinoline thiosemicarbazone (MAIQ). The mRNA levels for the small subunit of ribonucleotide reductase (RNR2) and sensitivity to hydroxyurea and MAIQ were determined in four strains of the yeast, *Saccharomyces cerevisiae*. Two strains exhibited significantly different sensitivities to both hydroxyurea and MAIQ, which closely correlated with differences in the levels of RNR2 mRNA. These results are consistent with recent observations with mammalian cells in culture, and indicate that a common mechanism of resistance to hydroxyurea and related drugs occurs through the elevation in ribonucleotide reductase message levels. A transplason mutagenized strain with marked structural modifications in RNR2 DNA and mRNA showed an extreme hypersensitivity to hydroxyurea but not to MAIQ, providing evidence that the two drugs do not inhibit the RNR2 subunit by the same mechanism. In addition, a yeast strain isolated for low but reproducible resistance to MAIQ exhibited a sensitivity to hydroxyurea similar to the parental wild-type strain, supporting the idea that the two drugs inhibit the activity of RNR2 by unique mechanisms. These yeast strains provide a useful approach for further studies into the regulation of eucaryotic ribonucleotide reduction and drug resistance mechanisms involving a key rate-timing step in DNA synthesis.

Dr. Arnold Greenberg and Postdoctoral Fellow, Dr. Shanti Samuel

LACK OF CORRELATION BETWEEN DEOXYRIBO-NUCLEOTIDE POOL SIZES, SPONTANEOUS MUTATION RATES AND MALIGNANT POTENTIAL IN CHINESE HAMSTER OVARY CELLS.

A.Y. Tagger, J.E. Damen, A.H. Greenberg, J.A. Wright

To examine the relationship between altered spontaneous mutation rates and malignant characteristics of cells, two hydroxyurea-resistant Chinese hamster ovary cell lines, with alterations in ribonucleotide reductase, were examined for their rates of spontaneous mutation to 6-thioguanine and ouabain resistance, tumor growth rates, and their ability to form experimental lung metastases. The most resistant cell line, H^R-R2T, showed no changes in the rate of spontaneous mutation to 6-thioguanine or ouabain resistance compared to the parental wild-type cell line; however, the mutant line formed lung metastases in experimental metastasis assays with BALB/c nu/nu mice, and exhibited metastatic abilities significantly different from the wild-type population. Furthermore, the H^R-R2T population did not show imbalances in any of the deoxyribonucleoside triphosphate pool sizes, which are frequently observed in cells altered in ribonucleotide reductase activity. The second hydroxyurea-resistant line, HN^R-AT, had gross alterations in dCTP and DGTP pools and although the rate of spontaneous mutation of 6-thioguanine resistance was unaltered, it showed a moderate decrease in the rate of spontaneous mutation to ouabain resistance when compared to the parental wild-type population. Interestingly, the HN^R-AT cell line did not form any lung metastases in the experimental metastasis assay. Both mutant cell lines H^R-R2T, and HN^R-AT, had increased tumor growth rates in C57 BALB/c "beige" nude (nu/nu) mice as compared to the parental wild-type population. In total, the results obtained with the two mutant cell lines question the association of altered mutation rates with increased metastatic potential. Although several explanations are possible for the altered malignant properties exhibited by H^R-R2T and HN^R-AT cells, it is interesting to note that the results are consistent with earlier suggestions that changes in ribonucleotide reductase may accompany modifications in the malignant characteristics of cells.

GENERATION OF METASTATIC VARIANTS IN POPULATIONS OF MUTATOR AND AMPLIFICATOR MUTANTS

J.E. Damen, A.Y. Tagger, A.H. Greenberg and J.A. Wright

Genetic instability has been hypothesized by P.C. Nowell and other investigators to be an important aspect of tumor progression that leads to the generation of metastatic variants in mutant cell lines having increased rates of spontaneous mutation and gene amplification. Parallel clonal populations of the spontaneous mutation rate mutant thy-49 and the gene amplification mutants YMP1 and YMP7 and their respective wild types were generated and grown to a critical population size. The number of metastatic variants in each clonal population was then determined following iv injection into nude mice. Lung tumors were scored 3-4 weeks after injection of cells, and the mean number per clonal population was determined. Analysis of the means with the Luria-Delbruck fluctuation test showed no significant differences in the rate of generation of metastatic variants produced in the genetically unstable lines compared to their normal counterparts. This study suggests that increased spontaneous mutation and gene amplification rates in mammalian cells

are not sufficient on their own to increase the rate of generation of metastatic variants.

STUDIES ON GLYCOSYL-TRANSFERASES IN FUSION-DEFECTIVE ConA-RESISTANT L6 RAT MYOBLAST CELL LINES

M.A. Spearman, S. Wayne, J.C. Jamieson, J.A. Wright and E. Gospodarek

As part of our studies on the regulatory roles played by glycoproteins on myoblast cell surfaces, we have investigated the activities of sialy-transferase and galactosyltransferase in wild type and conA-resistant variant cell lines. Sialyltransferase and galactosyltransferase are responsible for the attachment of the terminal and penultimate sugars, respectively, to complex type chains; moreover, these enzymes have not been studied in fusion defective myoblasts. We have made the following observations. 1) Sialyl- and galactosyl-transferase activities were determined in wild type and conA-resistant L6 rat myoblasts and substrates derived from fetuin, α-acid glycoprotein and bovine submaxillary mucin; fetuin was the best acceptor for both enzyme activities, whereas the mucin did not act as an acceptor. 2) The optimum pH for sialyltransferase was 6.6 in both cell lines. 3) The optimum pH for galactosyltransferase in the wild type cell line was 6.2 which was slightly higher than the value of 5.8 found for the conA-resistant cells. 4) Values for K_m for both enzyme activities increased five to ten-fold in the variant cell line with both acceptors. 5) The main sialyltransferase activity was the Galß1 rG1cNAcα2 3sialyltransferase for N-linked chains. The galactosyltransferase was most likely the enzyme that is responsible for the synthesis of the Galß1 4G1cNAc structure.

ONCOGENES AND METASTATIC PROGRESSION

A.H. Greenberg, S.E. Egan and J.A. Wright

It is now established that ras oncogenes can induce metastatic characteristics in primary diploid fibroblasts, nonsenescing fibroblasts and nonmetastasizing tumors. The issue of whether ras is directly involved in maintaining the metastatic phenotype through the expression and action of its gene product has been examined by analyzing the relationship to ras expression and to the production of the p21 ras-GTP complex, which is thought to mediate ras-transforming activity. While these expression and mutation studies support the idea that p21 ras directly regulates metastasis formation, it is also evident that there are many examples of human and murine cancers which show no differences in ras expression in primary and metastatic tumor cells. This may be partially explained by the ability of protein kinase-encoding oncogenes to also induce metastatic potential. In addition, the ability of ras to induce metastasis maybe dependent on the regulation of its activity by other genes. Furthermore, transformation does not occur as an isolated genetic event, but is rather the result of interaction of two or more oncogenes. We suggest that the nature of these gene interactions will ultimately determine whether a cell is a benign transformant or a malignant metastatic cancer.

HYDROXYUREA AND RELATED COMPOUNDS

J.A. Wright, G.A. McClarty, W.H. Lewis and P.R. Srinivasan

Hydroxyurea, a drug that has found use in clinical situations, is a specific inhibitor of DNA synthesis, and acts by inhibiting the activity of the highly regu-

lated enzyme ribonucleotide reductase. Hydroxyurea, and other drugs whose site of action is at one of the two non-identical subunits of ribonucleotide reductase (M1 or M2), have been used in cell culture to isolate drug resistant lines. These lines are useful for studying mammalian drug resistant characteristics, and the regulation of an exceptionally interesting enzyme which plays a central role in DNA synthesis, and therefore, cell proliferation. Hydroxyurea inactivates the M2 subunit by destroying a tyrosine free radical essential for activity, and cell lines resistant to hydroxyurea exhibit cross-resistance to other drugs which have a similar mode of action, such as guanazole and N-carb-amoyloxyurea. An interesting relationship between ribonucleotide reductase, hydroxyurea, and the commonly used chemotherapeutic agent bleomycin, has been observed. Drug resistant lines containing elevated levels of ribo-nucleotide reductase have been isolated, which exhibit collateral sensitivity to bleomycin. Furthermore, pretreatment of cells with hydroxyurea-related drugs rapidly converts the reductase to a form hypersensitive to bleomycin. The importance of ribonucleotide reductase in cell proliferation is stressed by observations suggesting that cell cultures altered in this activity can exhibit changes in proliferation-related characteristics. Molecular biology studies have indicated that hydroxyurea resistant lines frequently exhibit elevations in the M2 component, M2 mRNA, and M2 gene copy number. The most highly resistant lines may also possess chances in the M1 protein, which may or may not be accompanied by an amplification of the M1 gene. However, not all hydroxyurea resistant lines contain ribonucleotide reductase gene amplification, indicating that other molecular mechanisms regulating ribonucleotide reductase are involved in the resistance of some cell lines. Although the molecular studies are relatively recent, it is already clear that the drug resistant lines are capable of providing important new information about the regulation of ribonucleotide reduction, and about cellular mechanisms responsible for resistance to drugs whose target is at a key control point in the synthesis of DNA.

CO-REGULATION OF METASTATIC AND TRANSFORMING ACTIVITY OF NORMAL AND MUTANT ras GENES

S.E. Egan, J.J. Broere, L. Jarolim, J.A. Wright and A.H. Greenberg

We have analyzed the metastatic properties of NIH-3T3 cells transformed by H-ras activated through overexpression and/or mutation. Our results reveal that elevated expression of the H-ras proto-oncogene can induce the complete metastatic phenotype. Cells transformed by the proto-oncogene have a lower metastatic potential than cells transformed by a mutated ras gene. Ras oncogenes activated through alterations in codon 12 which encode p21 molecules with impaired GTPase activity, codon 59 which produce p21 molecules that release bound guanine nucleotides faster, or codon 61 which produce p21 having impaired GTPase activity and altered nucleotide release properties, are all able to induce the metastatic phenotype. Leucine 61 activated oncogenes with an additional mutation in codons 116, 117 or 119, resulting in a reduced affinity for guanine nucleotides, are also capable of inducing metastatic behaviour. These data indicate that ras genes which are capable of transforming are also capable of inducing the full metastatic phenotype in NIH-3T3 cells. This suggests that both phenotypes are induced through an increase in p21-GTP concentration in ras transformed cells. This established model for ras mediated transformation can also ex-

plain the qualitative and quantitative regulation of metastatic behaviour by ras.

MUTANT, NOT WILD TYPE, p53 COMPLEMENTS RAS IN THE FULL TRANSFORMATION OF REF52 CELLS

G. Hicks, S. Egan and M. Mowat

The rat embryo fibroblast line REF52 requires a second complementing oncogene to rescue it from the lethal growth arrest in G2 induced by transfection of the T24/EJ ras oncogene. This complementing activity can be contributed by SV40 large T, Adenovirus Ela, or, at a low frequency, c-myc gene products. We report here that an activated p53 immortalizing oncogene permits the establishment of stable ras transformed REF52 cells. Colonies isolated under co-selection for hygromycin resistance give rise to stable, ras expressing, transformed cell lines which are tumorigenic when injected subcutaneously in nude mice. To test whether this complementing activity is dependent on the expression of a mutant p53, we co-transfected a "wild type" full length genomic p53 gene molecularly cloned from the spleen of a BALB c nu/+ mouse. Results from preliminary studies show that this clone will not rescue ras transformed REF52 cells from growth arrest, while a full length genomic clone isolated from a Friend virus induced erythro-leukemic cell line, with previously demonstrated immortalizing activity will. Since we and others find p53 gene inactivation at a high frequency in transformed cells, we are currently investigating whether wild type p53 activity is insufficient for oncogenic comple-mentation or whether loss of wild type p53 activity can cooperate with ras in transformation of REF52 cells.

Dr. Michael Mowat and Doctoral students Wen Yang and Racquel Baert

ALTERED MAMMALIAN RIBONUCLEOSIDE DIPHOSPHATE REDUCTASE FROM MUTANT CELL LINES

J.A. Wright

From a biological, structural and regulatory point of view, ribonucleoside diphoshate reductase is one of the most complex enzymes in the cell. A well established approach to investigating a complex problem is the use of biochemical genetic techniques to compare the normal to the abnormal or altered situation. This approach to studying ribonucleoside diphosphate reductase is underway, and has yielded a variety of interesting mutant cell lines altered in the M1 and M2 components of the enzyme, and is contributing to our knowledge of key structural and regulatory aspects of this very interesting enzyme. Furthermore, the recent preparations of monoclonal antibodies and cDNAs specific for the two enzyme components have provided a powerful molecular approach for investigating the regulation of ribo-nucleotide reduction in normal and drug resistant mammalian cells. For example, these studies have indicated that hydroxyurea resistant lines frequently contain elevations in the M2 component, M2 mRNA, and M2 gene copy number. The most highly drug resistant lines may also possess changes in the M1 protein, which may or may not be accompanied by an amplification of the M1 gene. Although the molecular studies are relatively recent, it is already clear that drug resistant lines can provide important new information about the regulation of ribonucleoside diphosphate reductase, and about cellular mechanisms responsible for resistance to drugs whose target is a key control point in DNA synthesis. These biochemical and molecular studies indicate that multiple mechanisms involving the regulation of the two enzyme components are important determinants of chemotherapeutic sensitivity.

PUBLICATIONS:

Choy, B.K., McClarty, G.A. and **Wright, J.A.** Transient elevation of ribonucleotide reductase activity, M2 and mRNA and M2 protein in BALB/c 3T3 fibroblasts in the presence of 12-0-Tetradecanoylphorbol 13-Acetate. *Biochem. Biophys. Res. Commun.*, **162:**1417-1424, 1989.

Damen, J.E., Tagger, A.Y., Greenberg, A.H. and **Wright, J.A.** Generation of metastatic variants in populations of mutator and amplificator mutants. *J. Natl. Cancer Inst.*, **81:**628-631, 1989.

Damen, J.E., Tagger, A.Y., Greenberg, A.H. and **Wright, J.A.** Generation of metastatic variants in populations of mutator and amplificator mutants. *J. Natl. Cancer*, USA, **81:**628-631, 1989.

Egan, S.E., Broere, J.J., Jarolim, L., Wright, J.A. and **Greenberg, A.H.** Coregulation of metastatic and transforming activity of normal and mutant ras genes. *Int. J. Cancer*, **43:**443-449, 1989.

Egan, S.E., Spearman, M.A., Broere, J.J., Levy, A.B., Wright, J.A. and **Greenberg, A.H.** myc/ras cooperation is insufficient for metastatic transformation. UCLA Symposium on Molecular and Cellular Biology. *J. Cell. Biochem.*, **13B:**62, 1989.

Gingras, M.C., Wright, J.A. and **Greenberg, A.H.** Preferential survival of unique tumor subpopulations during lung implantation and invasion with altered matrix and protease gene expression. UCLA Symposium on Molecular and Cellular Biology. *J. Cell Biochem.*, **13B:**62, 1989.

Greenberg, A.H., Egan, S.E. and **Wright, J.A.** Oncogenes and Metastic Progression. *Invasion Metastasis*, **9**:360-378, 1989.

McClarty, G.A., Chan, A.K., Choy, B.K. and **Wright, J.A.** Increased ferritin gene expression is associated with increased ribonucleotide reductase gene expression and establishment of hydroxyurea resistance in mammalian cells. *J. Biol. Chem.* (in press), 1989.

Mowat, M., Sauder, M. and **Pereira, D.** The activated form of p53 is not a transactivator of the Intracisternal A Particle long terminal repeat promoter. *Oncogene*, **5**:241-244, 1990.

Rittenberg, D.A.H. and **Wright, J.A.** Relationships between sensitivity to hydroxyurea and 4-methyl-5amino-1 formylisoquinoline thiose micarbazone (MAIQ) and ribo-nucleotide reductase RNR2 mRNA levels in strains of *Saccbromyces cerevisiae*. *Biochem. Cell Biol.*, **67**:352-357, 1989.

Schwarz, L.C., Wright, J.A., Hall, A., Pimental, M., Chan, A., Sporn, M. and **Greenberg, A.H.** Enhanced secretion of activated TGF-ß and post receptor blockage to TGF-ß signaling in highly malignant fibrosarcomas. *Annals N.Y. Acad. Sci.* (in press), 1989.

Schwarz, L.C., Wright, J.A., Gingras, M.C., Kondaian, P., Danielpour, D., Pimentel, M., Sporn, M.B. and **Greenberg, A.H.** Abberant TGF-ß gene regulation in metastatic malignancy. *Growth Factors* (in press), 1989.

Spearman, M.A., Wayne, S., Jamieson, J.C., Wright, J.A. and **Gospodarek, E.** Studies on glycosyltransferases in fusion-defective con A-resistant L6 rat myoblast cell lines. *Int. J. Biochem.*, **21**:531-534, 1989.

Tagger, A.Y., Damen, J.E., Greenberg, A.H. and **Wright, J.A.** Lack of correlation between deoxyribo-nucleotide pool sizes, spontaneous mutation rates and malignant potential in Chinese hamster ovary cells. *J. Cancer Res. Clin. Oncol.*, **115**:429-434, 1989.

Wayne, S., Jamieson, J.C., Spearman, M.A. and **Wright, J.A.** Studies on the effect of Ketoconozole on the fusion of L6 myoblasts. *Mol. Cell. Biochem.* (in press), 1989.

Wright, J.A. Altered mammalian ribonucleoside diphosphate reductase from mutant cell lines. *Encyclopedia of Pharmacology and Therapeutics (Metabolic Inhibitors)*, **128**:89-111, 1989.

Wright, J.A., Smith, H.S., Watt, F.M., Hancock, M.C., Hudson, D.L. and **Stark, G.R.** DNA amplification is rare in normal human cells. *Proc. Natl. Acad. Sci.*, USA (in press), 1989.

Wright, J.A., McClarty, G.A., Lewis, W.H. and **Srinivasan, P.R.** Cellular resistance to hydroxyurea and related compounds. *In* Drug Resistance in Mammalian Cells. Vol. 1 *Antimetabolites and Cyto-toxic Analogues*, Edited by R. Gupta. CRC Press Inc., Boca Raton, Fl. pp.15-27, 1989.

PUBLISHED ABSTRACTS

Choy, B.K., McClarty, G.A. and **Wright, J.A.** Phorbol ester induction of ribonucleotide reductase in BALB/c 3T3 mouse fibroblasts. UCLA Symposium on Molecular and Cellular Biology *J. Cell Biocbem.*, **13B**:D305, 1989

Damen, J.E., Greenberg, A.H. and **Wright, J.A.** Rate of generation of metastatic variants in a mutator and two amplificator mutants. Critical Determinants in Cancer Progression and Metastasis, MD Anderson Cancer Center, pp. 125, 1989.

Greenberg, A.H., Schwarz, L.C., Gingras, M., Kondaiah, P., Danielpour, D., Sporn, M.B. and **Wright, J.A.** Aberrant TGF-ß gene regulation in metastatic malignancy. UCLA Symposium on Molecular and Cellular Biology, Negative Controls on Cell Growth. Taos, New Mexico, 1989.

Hicks, G., Egan, S., and **Mowat, M.** Mutant, not wild type, p53 complements ras in the full transformation of REF52 cells. Fifth Annual Meeting on Oncogenes, Frederick, Maryland, July, 1989.

Schwarz, L.C., Wright, J.A., Hall, A., Pimental, M., Chan, A., Sporn, M. and **Greenberg, A.H.** Enhanced secretion of activated TGF-ß and post-receptor blockage to TGF-ß signaling in highly malignant fibrosarcomas. Transforming Growth Factor-ßs: Chemistry, Biology and Therapeutics, New York, PI-22, 1989.

Wright, J.A., Hall, A., Pimental, M., Chan, A., Sporn, M. and **Greenberg, A.H.** Enhanced secretion of activated TGFß, and post receptor blockade to TGF-ß signaling in highly malignant fibrosarcomas. New York Academy of Sciences Meeting, Transforming Growth Factor-ß, Chemistry, Biology and Therapeutics. Washington, D.C., 1989.

III. BIOCHEMICAL PHARMACOLOGY

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ACTIVITY OF QUINONE ALKYLATING AGENTS IN QUINONE-RESISTANT CELLS

A. Begleiter and M.K. Leith

The role of the quinone group in the antitumor activity of quinone alkylating agents such as mitomycin C and 2,5-diaziridinyl-3,5-bis(carboethoxyamino)-1,4-benzoquinone is still uncertain. The quinone group may contribute to antitumor activity by inducing DNA strand breaks through the formation of free radicals and/or by influencing the alkylating activity of the quinone alkylators. The cytotoxic activity and DNA damage produced by the model quinone alkylating agents, benzoquinone mustard and benzoquinone dimustard, were compared in L5178Y murine lymphoblasts sensitive and resistant to the model quinone antitumor agent, hydrolyzed benzoquinone mustard. The resistant cell lines, L5178Y/HBM2 and L5178Y/ HBM10, have increased concentrations of glutathione and elevated catalase, superoxide dismutase, glutathione S-transferase, and DT-diaphorase activity. L5178Y/HBM2 and L5178Y/HBM10 cells were 7.4- and 8.5-fold less sensitive to benzoquinone mustard and 1.7- and 4.3-fold less sensitive to benzoquinone dimustard, respectively, compared to sensitive cells, but showed no resistance to the non-quinone alkylating agent, aniline mustard. The formation of DNA double strand breaks by benzoquinone mustard was reduced by 2- and 8-fold in L5178Y/HBM2 and L5178Y/HBM10 cells, respectively, while double strand break formation by benzoquinone dimustard was reduced only in the L5178Y/HBM10 cells. The number of DNA-DNA crosslinks produced by benzoquinone mustard was 3- and 6-fold lower, and the number produced by benzoquinone dimustard was 35% and 2-fold lower in L5178Y/HBM2 and L5178Y/HBM10 cells, respectively, compared to L5178Y parental cells. In contrast, crosslinking by aniline mustard was unchanged in sensitive and resistant cells. Dicoumarol, an inhibitor of DT-diaphorase, increased the cytotoxic activity of both BM and BDM in L5178Y/HBM10 cells. This study provides evidence that elevated DT-diaphorase activity in the resistant cells contributes to resistance to BM and BDM, possibly by decreasing the formation of the semiquinone intermediates of these agents. The altered reduction of the quinone groups in the resistant cells may be responsible for the decreased DNA-DNA crosslinking and lowered induction of DNA strand breaks by the quinone alkylating agents. These findings demonstrate that the quinone group can modulate the activity of quinone alkylating agents. The study also suggests that the semiquinone intermediates of BM and BDM may be the active alkylating species of these two agents.

THE QUINONE GROUP AS A MODULATOR OF ALKYLATING ACTIVITY IN QUINONE ALKYLATING AGENTS

A. Begleiter, M.K. Leith, and S. Pan

Previous studies have demonstrated that the quinone group may play an important role in modulating the alkylating activity of quinone alkylating agents. Introduction of a quinone moiety markedly increased the alkylating activity and cytotoxic activity of the model quinone alkylating agents, benzoquinone mustard and benzoquinone dimustard. However, the cytotoxic and DNA damaging activity of benzoquinone mustard

was considerably greater than that of benzoquinone dimustard. In this study, we have investigated the role of the quinone group as a modulator of alkylating activity in these antitumor agents using extracellular assays to eliminate differences due to cellular drug uptake and metabolism. Evidence was obtained that the alkylating activities of both benzoquinone mustard and benzoquinone dimustard were enhanced by reduction of the quinone group. In addition, when these agents were reduced, they showed nearly identical alkylating activity. This finding suggests that the difference in the activity of these agents in cells is not due to intrinsic differences in alkylating activities of the activated forms of these agents. Electrochemical studies revealed that benzoquinone dimustard has a lower redox potential than benzoquinone mustard and, thus is less easily reduced. Inactivation and spectroscopic studies suggested that the major reason for the differences in activity between benzoquinone mustard and benzoquinone dimustard may be the rapid inactivation of the dimustard prior to its reduction. This effect may be enhanced by the lower redox potential of benzoquinone dimustard compared to benzoquinone mustard. These findings support the hypothesis that the quinone group can modulate the alkylating activity of quinone alkylating agents; however, the mechanisms by which this modulation occurs may vary for different antitumor agents.

Dr. James Johnston

UPTAKE AND BINDING OF 3'-(3-CYANO-4-MORPHOLINYL)-3'-DEAMINOADRIAMYCIN AND 3'-(4-MORPHOLINYL)-3'-DEAMINOADRIAMYCIN IN L5178Y LYMPHOBLASTS IN VITRO

A. Begleiter, M.I. Jesson and J.B. Johnston

CMA and MA are analogues of Adriamycin with altered cytotoxic activity. CMA is 100 to 1500-fold more cytotoxic than Adriamycin and possesses unique DNA crosslinking activity. Although intact MA does not crosslink DNA and has cytotoxicity equivalent to Adriamycin, it retains its activity in anthracycline-resistant cells. In this study, uptake and binding of [^{3}H]CMA and [^{3}H]MA in L5178Y lymphoblasts was examined. Both CMA and MA were rapidly taken up by cells at 37°C and concentrated almost exclusively in the nucleus. All of the intracellular MA was TCA-soluble, but only 45% of this drug effluxed from the cells by 4 hr. Greater than 50% of CMA in the cells was TCA-insoluble and approximately 40% effluxed from the cells by 4 hr through loss of the TCA-soluble fraction. CMA differed from other alkylating agents in that greater than 97% of the bound drug was associated with DNA. The bound drug was partially lost from the DNA by a process that may involve DNA repair.

EFFECT OF TRANSFORMING GROWTH FACTOR-ß ON DNA SYNTHESIS IN B CELLS FROM CHRONIC LYMPHOCYTIC LEUKEMIA

L.G. Israels, L. Schwarz, A. Begleiter, M.R.A. Mowat, and J.B. Johnston

Transforming growth factor-ß (TGF-ß) is an important immunoregulator of human B and T lymphocytes as it down-regulates T cell clonal expansion, B cell proliferation and immunoglobulin secretion. Thus, it is probably an important negative regulator of lymphoid cell proliferation and function. In the present study we have found that in contrast to normal B cells, most leukemic cells from patients with chronic lymphocytic leukemia are either not inhibited, or are stimulated by TGF-ß. In addition there appears to be an inverse relationship between the rate of proliferation of the CLL cells and the effect of TGF-ß on there growth. This may in part account for the inexorable accumulation of these cells, as well as the immunosuppression common to this form of leukemia.

MECHANISMS INVOLVED IN INHIBITION OF REPAIR OF IRRADIATION INDUCED DNA STRAND BREAKS IN L5178Y LYMPHOBLASTS BY 2'-DEOXYCOFORMYCIN (dCF) AND DEOXYADENOSINE (dAdo)

J.B. Johnston, L. Verburg, L.G. Israels and A. Begleiter

We have previously observed a synergistic antitumor effect between irradiation and dCF/dAdo in resting L5178Y lymphoblasts and this was associated with an increase in cellular dATP and inhibition of repair of irradiation induced SB. In the present study, repair of DNA strand breaks was inhibited by dCF/dAdo in permeabilized resting L5178Y cells, and adenosine, which competed with dAdo for adenosine kinase, reversed this effect. However, repair was not inhibited by added dCF and dAMP, dADP, or dATP, which produced the same intracellular level of dATP as did dCF/dAdo. This indicates that the phosphorylation of dAdo may be critical for the inhibition of repair. Treatment of cells with dCF/dAdo, but not with dCF/dATP, resulted in a reduction in the intracellular levels of dGTP and dCTP. Whether a depletion of one or both of these deoxynucleotides, or an imbalance in the deoxynucleotide pools, is respon-

sible for the inhibition of repair is being investigated.

MECHANISMS OF RESISTANCE TO CHLORAMBUCIL IN CHRONIC LYMPHOCYTIC LEUKEMIA

A. Begleiter, L.G. Israels, M.R.A. Mowat and J.B. Johnston

We are investigating the biological and pharmacological mechanisms responsible for resistance to chlorambucil in chronic lymphocytic leukemia (CLL). We are studying the relationship between the clinical responses of CLL patients to chlorambucil and biochemical and pharmacological parameters in the leukemic cells isolated from these patients. To quantitate clinical responsiveness of CLL patients to chlorambucil, drug sensitivity is measured by the fall in peripheral lymphocyte count following a standard dosage of drug (0.9 mg/kg chlorambucil administered at 0.1 mg/kg 3 times daily for 3 days). Blood counts are measured on a weekly basis and clinical response is determined by the percent fall in lymphocyte count three weeks following drug treatment. This treatment regimen produces falls in lymphocyte counts at three weeks ranging from 0 - 93.6%.

Prior to chlorambucil treatment, the patients' leukemic cells are studied on two to three different occasions. Cells are incubated in vitro with 100 µM chlorambucil and DNA crosslink formation is measured by alkaline elution at 5, 10, 14 and 24 hours following addition of the drug. A number of pharmacological parameters are determined in these studies. Crosslinks at 5 hours represents a measure of the rate of crosslink formation; maximum crosslinks is a measure of the extent of crosslinking; crosslinks at 22 hours represents the level of crosslinks remaining following DNA repair, and area under the curve provides an overall measure of the number of crosslinks present in the DNA of the period of study. In addition, three parameters measuring the extent and rate of DNA crosslink repair are determined. The % repair is % of maximum crosslinks repaired at 22 hours; repair rate (RE/Hr) is the number of rad equivalents of crosslinks repaired per hour, and repair rate T^{1}_{2} is the time required to repair half of the maximum crosslink level.

We are also measuring a number of biochemical parameters within the leukemic cells of the CLL patients including: total sulfhydryl groups (TSH), non-bound sulfhydryl groups (NBSH), protein bound sulfhydryl groups (PSH), as well as the level of GSH, the major NBSH. Finally, total glutathione s-transferase activity (GST) is measured using 1-chloro-2,4-dinitrobenzine as a universal substrate.

We have now studied eighteen patients. The pharmacological and biochemical parameters for these patients have been correlated with the clinical response observed for these patients. None of the crosslinking or DNA repair parameters gave a statistically significant correlation with clinical response. Of these parameters, the best relationship was observed between DNA crosslinks at 22 hours and clinical responsiveness. Similarly, we did not observe a statistically significant correlation between any of the individual biochemical parameters and clinical response in these patients. There did appear to be an inverse relationship between the level of GSH in the cells and clinical response which approached statistical significance. However, when the GSH level was combined with the level of PSH either in the form of a ratio (PSH/GSH) or as a multiple regression, there was a significant correlation between these two parameters and clinical responsiveness. Interestingly, the level of PSH correlated directly with clinical response, while GSH showed an inverse correlation with response. In

contrast, there was no correlation between the level of GST activity in the leukemic cells and clinical response to chlorambucil treatment. These findings suggest that GSH may be involved in drug resistance and that PSH may serve as a target for chlorambucil. The apparent lack of correlation between PSH levels and clinical response may reflect a situation in which a specific subpopulation of PSH within the cell (either PSH at a particular cell location, or a specific protein sulfhydryl molecule) may represent a target for chlorambucil action. Studies to explore these possibilities are currently underway.

DIRECT CORRELATION BETWEEN DNA TOPOISOMERASE II ACTIVITY AND CYTOTOXICITY IN ADRIAMYCIN-SENSITIVE AND -RESISTANT P388 LEUKEMIA CELL LINES

D. Janendra, K. Batra and G.J. Goldenberg

The relationship between DNA topoisomerase II activity and drug resistance was studied in cloned cell lines of admiamycin (ADR)-sensitive and -resistant P388 leukemia; drug resistant P388/ADR/3 (clone 3) and P388/ADR/7 (clone 7) cells are 5- and 10-fold more resistant to ADR than the sensitive cell line P388/4 (Cancer Res., 46:2978, 1986). Topoisomerase Ii catalytic activity in crude nuclear extracts was reduced in drug-resistant cells as determined qualitatively by decatenation of kDNA. Using the centrifugal method of quantitative analysis, topoisomerase II catalytic activity (mean SE) was 81 10 units/mg total nuclear protein in sensitive cells, 29 2 units/mg total nuclear protein in resistant clone 3 cells, and 16 2 units/mg total nuclear protein in resistant clone 7 cells; these differences were highly significant ($P<0.005$). Similarly, restriction fragments showed that drug-stimulated topoisomerase Ii cleavage 2.9-fold greater than that from resistant clone 3 and 7 cells, respectively. Western blot analysis of nuclear extracts from the three cell lines using antibody against the C-terminal half of recombinant-prepared human topoisomerase II polypeptide revealed reduced immunoreactivity of topoisomerase II protein in the drug-resistant cells. These data suggest that reduced topoisomerase II activity unresistant cells, which may represent quantitative reduction of the enzyme, may be another property contributing to multifactorial drug resistance in these cells.

FURTHER CHARACTERIZATION OF DRUG-SENSITIVITY AND CROSS-RESISTANCE PROFILES OF CLONED CELL LINED OF ADRIAMYCIN-SENSITIVE AND -RESISTANT P388 LEUKEMIA

C. Seneviratne and G.J. Goldenberg

The drug sensitivity and cross-resistance profiles of cloned cell lines of Adriamycin-sensitive and -resistant P388 murine leukemia have been further characterized. A range of drug sensitivity that was more than 50,000-fold was observed for Adriamycin-sensitive cells; the most potent cytotoxic agent was the Adriamycin analog, 3'-(3-cyano-4-morpholinyl)3'-deamino adriamycin, and the least active compound was vinblastine. Adriamycin-resistant cells, which express the multidrug resistance phenotype, were cross-resistant to the DNA topoisomerase II interactive drugs: actinomycin D, daunorubicin, mitoxantrone, etoposide, and 4'(9-acridinyl-amino)methanesulfon-m-anisidide, to the vince alkaloids: vincristine and vinblastine, and to colchicine but not to the Adriamycin analog, 3'-(3-cyano-4-morpholinyl)-3'-deamino adriamycin or the alkylating agent, melphalan. These findings are consistent with other studies suggesting that 3'-(3-cyano-4-morpholinyl)3'-

deamino adriamycin acts as an alkylating agent. Studies with DNA topoisomerase II interactive agents, including mitoxantrone, the DNA intercalator, and etoposide, the epipodo-phyllotoxin showed that, as with adriamycin, cytotoxicity correlated closely with the formation of DNA double-strand breaks.

EVIDENCE FOR A MUTANT ALLELE OF THE GENE FOR DNA TOPOISOMERASE II IN ADRIAMYCIN-RESISTANT P388 MURINE LEUKEMIA CELLS

D. Bosman and G.J. Goldenberg

Previous studies have shown that DNA topoisomerase II enzyme activity and protein levels are reduced in cloned lines of Adriamycin-resistant P388 leukemia cells relative to drug-sensitive cells (Deffie, et al., Cancer Res., 49:58-62, 1989). The molecular basis of the reduced topoisomerase II levels in these resistant cells has been investigated. Northern blot analysis of total cellular RNA from drug-sensitive and -resistant cells using a 1.8 kilobase human topoisomerase II complementary DNA revealed the presence of two mRNA species: a 6.6-kilobase transcript that was strongly expressed in drug-sensitive cells but reduced 7-to 8-fold in resistant cells; and a 5.5-kilobase complementary DNA for human topoisomerase II showed that, in Adriamycin-resistant cells, there were two different alleles for topoisomerase II, one identical to the native allele but with a lower gene copy number than that found in sensitive cells, and a second allele containing a mutation present only in resistant cells. These findings suggest that the reduced levels of topo II protein in drug-resistant cells may be due to reduced amounts of the native 6.6-kilobase mRNA. The unique 5.5-kilobase mRNA in resistant cells may represent a shortened transcript of the mutated topoisomerase II allele.

DOWN-REGULATION OF PROGESTERONE RECEPTOR BY CALMODULIN ANTAGONISTS IN HUMAN BREAST CANCER CELLS

H-Y. Peter Lam and A.C. Chan

We have previously demonstrated that specific uptake of estradiol by MCF-7 cells was suppressed by calmodulin antagonists (AACR Proc. abstract 987,1988). In this report, we present evidence that the progesterone receptor (PR) level in human breast cancer cell line T47D is also down-regulated by calmodulin antagonists. Specific binding of [3H]-R5020 to T47D, which is a measure of PR in intact cells, plateaued after 1 hr and the level remained nearly constant for 3 hrs. Pretreatment of cells with W7, a calmodulin antagonist, at 50μM for 30min reduced the PR level to about 50% of control. This PR suppression effect lasted at least for 3 hrs after W7 treatment. Scatchard analysis of R5020 specific binding at 1 hr indicated a decrease in R5020 binding sites but no change in calmodulin antagonists showed that the extent of PR suppression correlated with the potency of the antagonists in the inhibition of calmodulin dependent cAMP phosphodiesterase: R24571 > 48/80 > TFP > W7 > W5. Interestingly, even when the highest non-cytotoxic concentrations fo the antagonists were used, about 35% of R5020 specific binding to T47D cells could not be suppressed. Competitive binding inhibition study of these calmodulin antagonists showed that they have little or no binding affinity for cytosolic PR; the relative binding affinity of these drugs were all below 0.001 while that of R5020 is defined as 100. Our results suggested that calmodulin is involved in the regulation of PR level in T47D cells; however, whether this effect is estrogen receptor-related remained to be determined.

PUBLICATIONS

Begleiter, A., Robotham, E., Lacey, G. and **Leith, M.K.** Increased sensitivity of quinone resistant cells to mitomycin C. *Cancer Lett.* **45**:173-176, 1989.

Deffie, A.M., Batra, J.K. and **Goldenberg, G.J.** Direct correlation between DNA Topoisomerase II activity and cytotoxicity in Adriamycin-sensitive and -resistant P388 leukemia cell lines. *Cancer Research*, **49**:58-62, 1989.

Deffie, A.M., Boxman, D.J. and **Goldenberg, G.J.** Evidence for a mutant allele of the gene for DNA topoisomerase II in Adriamycin-resistant P388 murine leukemia cells. *Cancer Res.*, **49**:6879-6882, 1989.

Johnston, J.B., Israels, G.J., Anhalt, C.D., Verberg, L, Mowat, M.R.A. and **Begleiter, A.** Glutathione S-transferase, sulfhydryl groups and DNA crosslinking activity with chlorambucil in chronic lymphocytic leukemia. *Journal of the National Cancer Institute*, **82**:776-779, 1990.

Rittberg, D.A.H., and Wright, J.A. Relationships between sensitivity to hydroxyurea and 4-methyl-5-amino-1-formylisoquinoline thiosemicarbazone (MAIQ) and ribonucleotide reductase RnR2 mRNA levels in strains of sacchromyces cerevisiae. *Biochem. Cell Biol.*, **67**:531-534, 1989.

Seneviratne, C. and **Goldenberg, G.J.** Further characterization of drug-sensitivity and cross-resistance profiles of cloned cell lines of Adriamycin-sensitive and -resistant P388 Leukemia. *Cancer Communications*, **1**:21-27, 1989.

Petkau, A., Lam, H-Y.P., Azzam, E.I., Ewing, D.D. and **Sargent, M.D.** Production and purification of mouse monoclonal antibodies to human Cu, Zn- and Mn-superoxide dismutases. Atomic Energy of Canada Ltd. Report 9909, 1989.

PUBLISHED ABSTRACTS

Begleiter, A., Jesson, M.I. and **Johnston, J.B.** Crosslinking activity of 3'(3-cyano-4-morpholinyl)-3'-deaminoadriamycin (CMA). *Proc. Amer. Assoc. Cancer Res.* **30**:558, 1989.

Deffie, A.M. and **Goldenberg, G.J.** Altered levels of expression of DNA Topoisomerase II in ADR-sensitive and -resistant P388 leukemia cells. *Proc. Am. Assoc. Can. Res.*, **30**:514, 1989.

Hurta, R.A.R., and **Wright, J.A.** Molecular and cellular characterization of drug resistant mouse cell lines with alterations in ribonucleotidereductase. *Proc. Can. Fed. Biol. Soc.*, **31**:75, 1989.

Jesson, M.I., Johnson, J.B., Robotham, E. and **Begleiter, A.** Characterization of the DNA-DNA crosslinking activity of 3'-(3-cyano-4-morpholinyl)-3'-deaminoadriamycin (CMA) *Proc. Amer. Assoc. Cancer Res.* **30**:491.

Johnston, J.B., Israels, L.G., Goldenberg, G.J., Mowat, M.R.A. and **Begleiter A.** Relationship between glutathione S-transferase (GST) activity, glutathione (GSH) levels, and DNA crosslinking (XL) activity with chlorambucil (CHL) in chronic lymphocytic leukemia (CLL). *Proc. Amer. Assoc. Cancer Res.* **30**: 514, 1989.

Johnston, J.B., Israels, L.G., Goldenberg, G.J., Mowat, M.R.A. and **Begleiter, A.** Relationship between glutathione S-transferase activity, glutathione levels and DNA crosslinking activity with chlorambucil in chronic lymphocytic leukemia. American Association for Cancer Research Meeting, San Francisco, May, 1989.

Leith, M.K., Robotham, E. and **Begleiter, A.** Increased activity of mitomycin C in quinone-resistant cells. *Proc. Amer. Assoc. Cancer Res.* **30**:558, 1989.

Lam, H.Y.P. and **Chan, A.** Down-regulation of progesterone receptor by Calmodulin Antagonists in human breast cancer cells. Proceedings of the American Association for Cancer Research Meeting, San Francisco, May 1989.

IV. IMMUNOLOGY AND HEMATOLOGY

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NATURAL ANTIBODIES

V. Miller, D. Chow and A. Greenberg

Natural antibodies are a ubiquitous and, in some ways, enigmatic group of antibodies. They appear to be phylogenetically primitive and have a wide range of distribution in vertebrate species from Agnathia to Mammalia, yet their origins and physiological role remain for the most part undefined. Their importance is suggested by the broad range of specificities recognised, including antigens on environmental pathogens, autologous and neoplastic cells. On the other hand, at the molecular level, they recognise common structural features such as specific carbohydrate sequences, alpha helices and phosphate esters, which may or may not have physiological or pathological significance. Much the same can be said of their reactivity with synthetic haptenes. This remarkable repertoire has been interpreted as a possible clue to their origin, which includes such hypotheses as autologous or environmental stimulation, and non-specific activation of B cells expressing autoreactive specificities determined by germ-line immunoglobulin V region sequences. At least some B cells expressing the Ly 1$^+$ phenotype produce natural antibodies and can therefore be distinguished from B cells producing immune antibody. This approach of identifying NAb-producing B cells by means of lineage markers, rather than on the basis of their specificity, may help clarify the origins of the NAb repertoire and the genetic control of NAb production. Indeed, a subclassification that distinguishes NAb originating from environmental stimulation from that appearing as a result of autologous activation may be possible with the Ly 1$^+$ B cell lineage. The heterogeneity of the B cell populations producing these antibodies undoubtedly has also led to the description of numerous and varied genetic patterns of regulation.

IDENTIFICATION OF A 40 Kd DENSE GRANULE MEMBRANE PROTEIN WHICH IS DEFICIENT IN A PATIENT WITH THE HERMANSKY-PUDLAK SYNDROME

J.M. Gerrard, D. Lint, S.J. Israels,* P.J. Sims*, T. Wiedmer, T.*, and D. Fugate*

A series of monoclonal antibodies were prepared to platelet dense granules following immunization of mice with a purified dense granule fraction prepared from sonicated washed human platelets pelleted through 40% metrizamide. Several antibodies were obtained which recognized a 40 Kd protein (by Western blot), found specifically in the membrane of the platelet dense granule. The protein was not released from the granule membrane by sonication, but was readily solubilized in 0.2% lubrol. Western blots and slot blots confirmed the protein was markedly (50-100 fold) enriched in the dense granule preparation compared to the whole platelet homogenate. Immunofluorescence punctate spots inside the platelets using two separate antibodies to the 40 Kd protein. The appearance of these spots was similar to spots obtained in incubating platelets with quinacrine although slightly fewer in number (4.55 ± 0.35 spots per platelet). Platelet whole mounts evaluated by electron microscopy showed 2.62 ± 0.54 dense granules per platelet in the same platelet samples. Studies of a patient with the Hermansky-Pudlak syndrome (a deficiency of dense granule contents associated with albinism) showed 0.54 and 0.55 ± 0.23 dense granules per platelet using two antibodies to the 40 Kd protein, and 1.84 ± 0.2 dense granules per

platelet using quinacrine. Quantitation (by slot blot) of the amount of the 40 Kd protein in the platelets from the patient with the HP syndrome showed approximately 10% of the level found in 5 normal controls.

NEONATAL PLATELET FUNCTION: EVALUATION OF DENSE GRANULE SECRETION

Sara J. Israels and Michele Daniels

The secretion response of neonatal platelet dense granules has previously been shown to be abnormal, however, the basis for this abnormality has not been elucidated. This secretion response in human platelets, regardless of the initial agonist, is the result of activation of two complementary, but separable pathways: Calcium-dependent granule centralization and protein kinase C-dependent granule fusion. Using specific activators of each pathway, inositol-1,4,5-trisphosphate (IP3, 10 μM) to stimulate intracellular calcium flux, and oleyl-acetyl glycerol (OAG, 100 μg/ml) to stimulate protein kinase C activation, we have evaluated dense granule secretion and phosphorylation of two platelet proteins in neonatal platelets and adult controls. We also evaluated the effects of two physiologic agonists, thrombin (0.2 U/ml) and soluble bovine collagen (1 μg/ml), which activate both pathways. Neonatal platelets do not differ from adult blood platelets in their response to thrombin, IP3 or OAG for secretion of (14C)-serotonin or phosphorylation of myosin light chain and 47,000 dalton protein. Neonatal platelets, however, failed to show either a secretion or a protein phosphorylation response to collagen stimulation. These results suggest that the complementary pathways resulting in secretion are intact in the neonatal platelet, but that an earlier activation step may be deficient, particularly one that is critical to collagen-induced activation.

LOW NATURAL ANTIBODY AND LOW IN VIVO TUMOR RESISTANCE, IN XID-BEARING B-CELL DEFICIENT MICE

Donna A. Chow and Rita D. Bennet

Evidence from correlative studies and Winn-type assays in syngeneic murine models has suggested that natural antibodies contribute to resistance against tumors in vivo. The B cell deficit associated with the x-linked immunodeficiency of CBA/N strain mice provided a genetic model in which to further test this question. RI-28, a radiation-induced T cell leukemia of the CBA/H strain acquired reduced levels of fluorescence-detected natural antibodies from the serum of X-linked immunodeficiency-bearing CBA/N and male (CBA/N x CBA/J)F1 mice compared with the serum from normals. Threshold s.c. inocula of the RI-28 appeared sooner and produced higher tumor frequencies in the X-linked immunodeficiency-bearing animals. This data coupled with the lack of correlating deficiencies in natural killer cell or activated macrophage activity provide the first genetic evidence for the hypothesis.

INVERSE CORRELATION BETWEEN NATURAL ANTI-TUMOR ANTIBODIES AND TUMOR SUSCEPTIBILITY IN INDIVIDUAL XID-BEARING MICE

R.D. Bennet and D.A. Chow

Natural antibodies, natural killer cells and activated macrophages, have all been implicated in the rejection of threshold syngeneic tumor inocula. Previous analysis of tumor susceptibility in normal vs. inbred and F1 mice bearing the B-cell deficiency associated with the xid-mutation of CBA/N mice demonstrated an inverse relationship between the tumorigenicity of the RI-28 serum NAb levels in mice with the same genetic origins. No relationship with tumor susceptibility was seen with NK cell or in

vivo activated macrophage cytolysis. Flow cytometry determination of anti-tumor serum NAb bled from individual male and female (CBA/N x CBA/J) F1 mice one week prior to the threshold tumor inoculation has revealed extensive heterogeneity within the NAb levels of each sex. A comparative analysis of tumor fate with NAb activity revealed that tumors appeared in only 26.3% of animals with a mean fluorescence channel binding above 60 channels in contrast with 77.3% of animals with lower NAb levels. These data extend to the level of individual hosts the support for an inverse relationship between host NAb activity and tumor susceptibility. In addition, subsequent analysis of serum anti-tumor NAb levels, splenic NK cytolysis and in vitro lymphokine activated macrophage activity with all three mediators originating from the same individual F1 mice showed no consistent correlations among these natural resistance activities arguing for the exclusion of deficiencies in NK cell or macrophage function as the basis for the differential tumor susceptibility in individual F1 mice.

RNK GRANULE EXTRACT CYTOLYSIS: DIFFERENTIAL INHIBITOR PRODUCTION BY AN NK-RESISTANT VS AN NK-SENSITIVE MURINE LYMPHOMA

Donna A. Chow

Natural killer (NK) cell-resistant tumors exist despite their ability to bind cells from the effector population. Tumor sensitivity to NK activity was therefore examined at the level of susceptibility to cytolysin-containing NK cell cytotoxic granule extracts. The NK-sensitive SL205 murine lymphoma was markedly more susceptible than the NK-resistant L5178Y-F9 to solubilized granule preparations from the rat NK tumor cell line RNK-16, and this corresponded also with tumor sensitivity to hypotonic lysis. However, the resistant L5178Y-F9 was better able to inhibit the extract activity than the SL2-5. Dissociation of the binding and lysis phases of the cytolysin reaction based on their differential temperature requirements, 4°C for binding and 37°C for lysis, permitted an examination of the cytolysin/tumor interaction prior to lysis. The residual cytotoxic activity was lower after extract exposure to the L5178Y-F9 compared with the SL2-5 consistent with possible inhibitor production. Finally, supernatant material collected from the L5178Y-F9 was a better inhibitor of granule extract lysis and acted preferentially in the extract-binding phase. The inhibitor appears to be protein in nature, relatively stable and exhibits molecular weight heterogeneity ranging from 2000 to greater than 300,000.

INTRACELLULAR HISTAMINE SYNTHESIS IS CRITICAL FOR COLLAGEN INDUCED PLATELET AGGREGATION

S.P. Saxena, A. McNicol, L.J. Brandes, A.B. Becker, and J.M. Gerrard

Recently, we reported evidence suggesting that newly-formed histamine is an intracellular messenger which promotes aggregation and other platelet responses (Blood 72:331a, 1988). We have further investigated stigated the role of histamine in physiological activation of platelets by collagen. Histamine was analyzed by HPLC equipped with a fluorometric detector. Human platelets stimulated with collagen (1.8 μg/ml) synthesized up to 21 pmoles histamine/10^9 platelets by 2 min.(or 2.9 μM intracellularly). Histamine production was not dependent on aggregation since the cyclooxygenase inhibitor, indo-methacin (10μM; 15 min.) and the novel intracellular antagonist, N, N-dimethyl-2-[4-(phenylmethyl)-phenoxy] ethanamine.HC1 (DPPE) (10μM; 30 sec.) inhibited collagen-induced platelet ag-

gregation but did not suppress the increase in newly-formed histamine. Furthermore, histamine production was normal in platelets from a patient with defective aggregation due to a dense granule deficiency. In contrast, histidine decarboxylase (HDC) inhibitors α-methyl histidine (α-MH) and α-fluoromethylhistidine (α-FMH) inhibited both collagen-induced histamine production and platelet aggregation in parallel. Studies with α-FMH were particularly revealing in that a time-dependent deactivation of HDC, similar to that reported in other tissues, was observed in platelets. The IC^{50} of α-FMH for histamine production and aggregation was 600μM and 5μM for 30 sec. and 120 minute preincubation, respectively. The inhibitory effects of DPPE and the HDC inhibitors on collagen-induced platelet aggregation were significantly reversed by the addition of histamine (100nM - 10μM) to saponin- permeabilized platelets; histamine alone had no pro-aggregatory effects. Furthermore, DPPE (10μM) inhibited collagen-induced thromboxane production and serotonin secretion by approximately 50% and 90%, respectively. These inhibitory effects were also reversed by the addition of histamine. These studies suggest that synthesis of newly-formed histamine is critical for collagen-induced platelet activation with aggregation being the end result of several synergistic mechanisms, one of which involves histamine.

HISTAMINE SYNTHESIS AND FUNCTION IN ACTIVATED PLATELETS: SIMILARITY OF ACTIVATION MESSAGES INVOLVES IN SIGNAL TRANSDUCTION IN PLATELETS TO THOSE FOUND DURING CELL PROLIFERATION

J.M. Gerrard, S.P. Saxena, A.B. Becker, F.S. LaBella, and L.J. Brandes

Signal transduction processes involved in the stimulation of platelets have similarities to those found during the initiation of cell proliferation including altered phospholinositide metabolism, a rise in cytosolic calcium and activation of protein kinase C. Our recent studies suggest an additional similarity between platelet activation and the regulation of cell growth. Histamine production is high in rapidly growing cells and tissues including fetal tissues, healing wounds and cancerous tissues. The induction of histidine decarboxylase (HDC), the enzyme responsible for histamine production, and the synthesis of histamine in these tissues was first suggested to play a role in cell growth by Kahlson in 1960. Since then, additional evidence supporting a possible role of histamine in growth has accumulated including studies which show that inhibition of histamine synthesis, or antagonism of histamine at a novel intracellular histamine receptor (HIC) is associated with decreased cell growth. We now report that platelets stimulated by thrombin, collagen, platelet activating factor and phorbol-12-myristate-13-acetate (PMA) show a burst of newly-formed cytoplasmic histamine in association with platelet activation. The importance of this newly-formed histamine is suggested by studies showing inhibition of PMA and collagen-induced platelet aggregation by HDC inhibitors and the H_{IC} receptor antagonist, N,N-dimethyl-2-[4-(phenyl-methyl) phenoxy]ethanamine.HC1(DPPE), and the reversal of such inhibition by addition of histamine (10^{-7} to 10^{-5}) to saponin-permeabilized platelets. The results suggest that activation of platelets and initiation of cell growth may share a common pathway mediated by changes in intracellular histamine, and imply a widespread, fundamental and novel role for this amine in cellular physiology.

Dr. Sara Israels

A ROLE FOR INTRACELLULAR HISTAMINE IN COLLAGEN-INDUCED PLATELET RESPONSES

S.P. Saxena, A. McNicol, L.J. Brandes, A.B. Becker and J.M. Gerrard

Previously we reported that newly-formed intracellular histamine is a mediator of phorbol-12-myristate-13-acetate (PMA)-induced platelet aggregation through binding to a novel site of μM affinity, designated as HIC. To further investigate the role of histamine in platelet responses, we measured its production in response to a more physiological agonist collagen. Histamine was analyzed using HPLC equipped with a fluorometric detector. Platelets stimulated with collagen (1.8 μg/ml) were found to synthesize up to 39 pmol of newly-formed histamine per 10^9 platelets. Platelet aggregation in response to collagen was inhibited by a-methyl histidine (a-MH), a histidine decarboxylase inhibitor (IC_{50} = 750 μM), a concentration similar to that required to inhibit platelet histamine production. A specific antagonist for HIC, N, N-dimethyl-2-[4-phenylmethyl) phenoxy]ethanamine.HCl (DPPE), inhibited collagen-induced platelet aggregation (IC_{50} = 6 μM). DPPE also inhibited collagen-induced serotonin secretion and thromboxane formation. Addition of histamine (1-10 μM) to saponin-permeabilized platelets reversed the inhibitory effects of a-MH and DPPE on platelet aggregation, serotonin secretion and thromboxane formation. These results show that newly-formed histamine is an intracellular messenger mediating the effects of collagen in platelets. Supported by MRC Grant MA7396.

ENDOTHELIUM-DERIVED RELAXING FACTOR (EDRF) INHIBITS INTRALUMINAL PLATELET AGGREGATION, BUT IS BLOCKED BY RED BLOOD CELL (RBS) HAEMOGLOBIN (Hb)

D.S. Houston and J.M. Gerrard

Studies were done to determine whether EDRF release can inhibit platelet aggregation within the vascular lumen, and whether the inhibition is reversed by RBCs. Canine femoral arteries mounted in an organ bath were perfused with Krebs solution. During contraction with phenylephrine $3x10^{-6}$ M, acetylcholine 10^{-6} M (ACh), bradykinin 10^{-6} M (Bk) or thrombin 1 U/ml caused relaxation of the vessel wall (measured by hooks connected to a force transducer). The relaxation to thrombin was tachyphylactic. Washed human platelets labelled with ^{14}C-5HT were added to the perfusing solution, and activated by thrombin 0.25 U/ml infused via a branch vessel. The perfusate which was collected below and centrifuged; the fraction of ^{14}C-5HT appearing in the supernatant reflected the degree of platelet activation. Stimulation of EDRF release with ACh or Bk inhibited ^{14}C-5HT release (by 18 ± 8% with ACh). Both relaxation and platelet-inhibition were blocked by Hb 10^{-5} M. [ACh, Bk and Hb did not directly affect platelet aggregation or ^{14}C-5HT release in an aggregometer.] RBCs (treated with echothiophate 10^{-6}M to block

RBC cholinesterase, then washed) did not inhibit relaxation but reversed the platelet-inhibition at a hematocrit of 10%, but not 1% or 5%. Thus, RBC haemoglobin may regulate inhibition of intraluminal platelet aggregation by EDRF.

Dr. Frixos Parashevas and technicians, Margaret Chapiel and Kathy Ulicki

HISTAMINE PRODUCED INTRACELLULARILY IN RESPONSE TO COLLAGEN ACTS TO PROMOTE AGGREGATION AT A STEP INDEPENDENT OF, OR DISTAL TO, PHOSPHOINOSITIDE METABOLISM AND INCREASED CYTOSOLIC CALCIUM

A. McNicol, S.P. Saxena, L.J Brandes and J.M. Gerrard

Recent studies suggest a role for histamine (HA) as an intracellular messenger in human platelets activated by collagen. HA is synthesised in collagen stimulated platelets. Platelet aggregation and the formation of HA are both prevented by inhibitors of histidine decarboxylase (HDC). In addition the novel intracellular HA antagonist N, N-dimethyl-2-[4-(phenylmethyl) phenoxylethanamine (DPPE) attenuates collagen-induced platelet aggregation without affecting HA formation. We have examined whether newly formed HA is required for phosphoinositide metabolism and the elevation in cytosolic free calcium levels ($[Ca^{2+}]_i$) in platelets challenged with collagen. In plasma free suspensions of human platelets aggregation was monitored photometrically, $[Ca^{2+}]_i$ was measured by Fura 2 fluorescence and phosphoinositide metabolism assessed by the formation of [^{32}P]-phosphatidic acid (PA) in ^{32}P-orthophosphate prelabelled platelets. Collagen (1.8 µg/ml) stimulated aggregation, elevated $[Ca^{2+}]_i$ (from 99±3 to 211±16 nM) and induced the formation of [^{32}P]-PA (by approximately 7 fold). Preincubation with concentrations of the HDC inhibitors a-methylhistidine (1mM; 30s) or a-fluoromethylhistidine (100 µM; 120 minus.) sufficient to block HA formation also inhibited collagen induced aggregation but had no significant effect on either [^{32}P]-PA production or the elevation of $[Ca^{2+}]_i$ (to 221±21 nM and 246±32 nM respectively). DPPE (25µM; 30s) inhibited collagen-induced aggregation but

Research Associate, Dr. Lianfa Shi

had no effect on the increase in $[Ca^{2+}]_i$ (to 208±40 nM) or on $[^{32}P]$-PA formation.

The results show that newly formed intracellular HA acts after or independent of, though possibly synergistically with, the phosphoinositide/calcium mediated mechanism of collagen induced platelet activation.

EVIDENCE THAT INTRACELLULAR HISTAMINE HAS A MAJOR ROLE IN COLLAGEN BUT NOT IN THROMBOXANE ACTIVATION OF PLATELETS

A. McNicol, S.P. Saxena, L.J. Brandes and J.M. Gerrard

Collagen-induced platelet activation is associated with the production of both thromboxane A_2 (TxA_2) and intracellular histamine (HA). Both TxA_2 and HA appear to be critical to platelet activation by low concentrations of collagen. We have now attempted to clarify the role of HA with particular reference to TxA_2. Preincubation (30s) of the platelets with either the intracellular HA antagonist, N,N-dimethyl-2-[4-(phenylmethyl) phenoxy]ethanamine.HCl (DPPE)(1-100 µM) or the histidine decarboxyase inhibitor α-methylhistidine (α-MH)(1µM-5 mM) markedly inhibited collagen (1.8 µg/ml) induced aggregation and ^{14}C-serotonin secretion, but had little effect on that stimulated by the TxA_2 analog EP171 (5 nM). The addition of HA (0.1-1 µM) to saponin permeabilized platelets reversed these inhibitory effects on collagen responses but had little effect to promote responses to EP171. The collagen-induced increase in cytosolic-free calcium levels ($[Ca^{2+}]_i$), as monitored by Fura 2, was inhibited by indomethacin, DPPE, α-MH, and PGI2. In contrast, only PGI_2 significantly altered the effects of EP171 on $[Ca^{2+}]i$. The production of TxA_2, as assessed by ELISA, in response to collagen, was inhibited by DPPE while collagen-induced HA formation was little affected by aspirin. These results indicate that HA has a major role in collagen, but not in TxA_2-induced platelet activation suggesting that HA acts, at least in part, before TxA_2 formation.

LOW NATURAL ANTIBODY AND LOW IN VIVO TUMOR RESISTANCE, IN XID-BEARING B-CELL DEFICIENT MICE

D.A. Chow and R.D. Bennet

Evidence from correlative studies and Winn-type assays in syngeneic murine models has suggested that natural antibodies contribute to resistance against tumors in vivo. The B cell deficit associated with the x-linked immunodeficiency of CBA/N strain mice provided a genetic model in which to further test this question. RI-28, a radiation-induced T cell leukemia of the CBA/H strain acquired reduced levels of fluorescence-detected natural antibodies from the serum from normals. Threshold s.c. inocula of the RI-28 appeared sooner and produced higher tumor frequencies in the X-linked immunodeficiency-bearing animals. This data coupled with the lack of correlating deficiencies in natural killer cell or activated macrophages activity provided the first genetic evidence for the hypothesis.

THE ISOTOPE OF AN Ig CONTAINING MEDIATOR DICTATES THE ISOTOPE OF THE ANTIBODY PRODUCED: A NOVEL MECHANISM OF ISOTOPE REGULATION

S.T. Lee, J. Maeba, P.B. Ernst, J. Bienenstock and F. Parasheuas

Within 6 hours after primary immunization the serum of mice contains a unique form of processed antigen which

is complexed with immunoglobulin (Ig). These complexes are formed through the mediation of a T cell factor and markedly enhance in vivo the 7s antibody response. They possess potent anti-suppressor activity, i.e., they reverse suppression to immunity both in vivo and in vitro. We have been able to generate in vitro complexes containing a single Ig isotope. Such complexes returned the antibody formation in a suppressed culture for only the same Ig subtype as that present in the complexes. Thus, complexes containing IgG2a, induced exclusively IgG2a antibody and those containing IgA returned only the IgA response in the suppressed culture. The selection of a particular isotope for the formation of the complexes is determined by inducer T cells that produce the factor. The spleen contains inducer T cells capable of mediating the formation of complexes with IgG2a or IgA, while the T cells from Peyer's patches can induce the formation only of IgA complexes. The formation of the complexes is the earliest response to antigen in vivo and, according to the data presented here, the type of isotopes to be synthesized during an immune response may be determined as early as 3-6 hours after immunization as a result of the formation of these isotope specific complexes.

IDENTIFICATION OF A MACROPHAGE-ACTIVATING FACTOR IN GRANULES OF RNK LARGE GRANULAR LYMPHOCYTE LEUKEMIA

E. Roussel and A.H. Greenberg

Recent work from our laboratory has shown that NK cells rapidly release performed factor(s) that stimulate monocyte oxidative metabolism and microbicidal activity. We have hypothesized that such factors could also activate macrophage (M) tumor lysis and might be stored in the cytoplasmic granules. Granules were isolated from the RNK large granular lymphocyte leukemias by nitrogen cavitation and Percoll fractionation of the cell homogenate. Utilizing CSF-1 differentiated murine bone marrow-derived M and P815 tumor target cells, a M -activating factor (MAF) was found. The MAF activity was identified in two peaks, the first was coincident with dense granule enzymes and was 60 times more concentrated per mg protein than a second peak in the cytosol fractions. Solubilization in 2 M NaCl was necessary to recover activity from both peaks. Granule NK-MAF required the simultaneous presence of LPS in order to induce tumoricidal activity. Kinetics of NK-MAF activation peaked after 12 h of exposure. The NK-MAF was short lived in the solubilized granules; however, its heat resistance allowed us to prepare enriched and stable preparations. Treatment of NK-MAF with μepsin but not with a nominal cut-off of 10 kDa. This work indicates that NK cell granules contain a small heat-stable peptide capable of activating M tumoricidal activity.

A ROLE FOR ISOTOPE-SPECIFIC BINDING FACTORS IN THE REGULATION OF Iga- and IgG-SPECIFIC RESPONSES BY THE ANTI/CONTRASUPPRESSOR T CELL CIRCUIT

P.B. Ernst, S. Tone-lee, J. Maeba, J. Bienenstock, A. M. Stanisz and F. Paraskevas

Previous studies have shown that the isotope of an antibody response is selected, in part, by the inhibition of isotope-specific suppression. The antisuppressor model predicts that isotope selection is initiated through an interaction between Ag, Ig, and a T cell-

Dr. Lorne Brandes

derived factor within 6 h of immunization. This report characterizes some of these molecules and their contribution to isotope regulation. Cultures of murine spleen cells stimulated with the T cell-dependent Ag SRBC led to Ag-specific IgG and IgA responses that could be suppressed and then antisuppressed by a molecular complex produced by mixing purified serum Ig with the supernatant of Ag-pulsed macrophages and rIL-1α stimulated CD4+ T cells, could be pooled and mixed with Ig to produce functional antisuppressive complexes thereby allowing the factors from the different cell types to be studied separately. Adsorption of the co-culture or the rIL-1α stimulated T cell supernatants against monoclonal IgG or IgA, removed IgG and IgA binding factors, respectively, and abrogated the ability to enhance the corresponding isotope. The adherent material could be recovered and used to reconstitute enhancement by the supernatants depleted of the binding factors. When affinity purified IgG or IgA was used as the source of Ig within the antisuppressive complexes, the enhancement of the antibody response was limited to the isotope of the regulatory Ig used to form the complex. Thus, manipulation of the antisuppressive molecules has a predictable effect on isotope selection. Release of isotope-specific binding factors by CD4+ cells by rIL-1α supports the hypothesis that T cell circuits play a role in initiating isotope regulation.

NATURAL KILLER CELL REGULATION OF MURINE EMBRYONIC PULMONARY FIBROBLAST SURVIVAL IN VIVO

N. Khalil and A.H. Greenberg

We examined the role of the natural killer (NK) cell in controlling the survival of embryonic pulmonary fibroblasts in vivo. In vitro, both primary embryonic fibroblasts and an embryonic fibroblast line (10T 1/2) were lysed by syngeneic C3H/HeN splenocytes threefold more efficiently than primary adult fibroblasts. The membrane phenotype of the effector cells was typical of NK cells. It was asialo GM1$^+$, Lyt 2,1-, Lyt 1.1-, Thy 1.2-. The cytotoxicity of the effector cell could be enhanced by IFN-α/β but was deficient in the C3H/H3J bg/bg mutant. Iododeoxyuridine (^{125}I-dUrd)-labeled embryonic fibroblasts were injected intravenously into syngeneic mice with either enhanced or deficient NK function and their survival in the and their survival in the lung was quantitated. Enhanced fibroblast survival was detected in the NK deficient C3H/HeJ beige (*bg/bg*) mutant strain compared to its normal littermate C3H/HeJ (*bg/bg*). A second method of NK depletion by pretreatment with rabbit anti-asialo GM1 antiserum also produced a striking increased in fibroblast survival. Poly(I:C) significantly enhanced the elimination of pulmonary fibroblasts from the lung between 4 and 24 hr after injection. Poly(I:C) did not enhance clearance of pulmonary fibroblasts in the C3H/HeJ (*bg/bg*) mutant, but did so in the normal littermate C3H/HeJ (*bg/+*). In conclusion, we have shown that the survival of embryonic pulmonary fibroblasts was inversely correlated with in vivo NK activity suggesting a possible role for this cytotoxic cell in the control of fibroblast growth *in vivo*.

MACROPHAGE PRODUCTION OF TRANSFORMING GROWTH FACTOR ß AND FIBROBLAST COLLAGEN SYNTHESIS IN CHRONIC PULMONARY INFLAMMATION

N. Khalil, O. Bereznay, M. Sporn, A.H. Greenberg

A rat model of bleomycin-induced pulmonary inflammation and fibrosis was used to examine the relationship between collagen synthesis and transforming growth factor ß (TGF-ß) production, and cellular distribution. Total lung TGF-ß was elevated within 2 h of intratracheal bleomycin administration and peaked 7 d later at levels 30-fold higher than controls. This was followed by a gradual decline with lower but persistent levels of production in the late phase of the response between 21 and 28 d later. The peak TGF-ß levels preceded the maximum collagen and non-collagen protein synthesis measured by [3H]proline incorporation into lung fibroblast explants of bleomycin-treated rats. The pattern of immunohistochemical staining localized TGF-ß initially in the cytoplasm of bronchiolar epithelium cells and subepi-thelial extracellular matrix. The peak of lung TGF-ß levels of TGF-ß levels at 7 d coincided with intense TGF-ß staining of macrophages dispersed in the alveolar interstitium and in organized clusters. Later in the course of the response, TGF-ß was primarily associated with extracellular matrix in regions of increased cellularity and tissue repair, and coincided with the maximum fibroblast collagen synthesis. This temporal and spatial relationship between collagen production and TGF-ß production by macrophages suggests an important if not primary role for TGF-ß in the pathogenesis of the pulmonary fibrosis.

DRUG-INDUCED (POLY I:C) PYREXIC RESPONSES: CONGRUENCE WITH A COMPENSATORY CONDITIONING ANALYSIS

D. Dyck, T.A.G. Osachuk, A.H. Greenberg

Preliminary experiments indicated that DBA/2J mice exhibit a pyrexic response 4-6 h after injection with the synthetic, double-stranded polynucleotide, polyinosinic polycytidylic acid (poly I:C), a macrophage and natural killer (NK) cell activating compound. We tested the hypothesis that a procedure involving repeated paired delivery of odor cues and poly I:C would result in the elicitation of a conditioned "drug-compensatory" hypothermic response to a cued placebo injection. A 2 x 2 factorial design was employed in a two-phase experiment with mice. In the first phase, two groups of mice were given four weekly, signaled injection of either poly I:C (50µg/mouse) or saline. Rectal temperature was taken immediately prior to the injection and at 1-h intervals for the next 6 h. During the second 4-week phase, half of the mice in the saline (S) and poly I:C (P) groups were maintained on the same drug regimen (S-S; P-P); the other half were shifted to the opposite drug condition (P-S; S-P). The results indicated that the mice given paired cue-drug delivery in Phase 1 demonstrated a robust hypothermic response to cued placebo injection in Phase 2 (Group P-S). This drug-compensatory hypothermic response was evident over all 4 weeks of testing. These results are consistent with the interpretation that drug-compensatory conditioning occurred to cues paired with poly I:C, and, more generally, our findings support the hypothesis that the effects of this immunostimulatory agent are subject to modification by the central nervous system.

PUBLICATIONS

Atkinson, E.A., Gerrard, J.M., Hildes, E.A., Greenberg, A.H. Production of inositol-phospholipid-derived second messengers in a rat natural killer cell line exposed to susceptible tumor targets. *Nat. Immun. Cell Growth Regul.* **8:**223-230, 1989.

Bennet, R.D., and **Chow, D.A.** Inverse correlation between natural anti-tumor antibodies and tumor susceptibility in individual xid-bearing mice. *Natural Immunity and Cell Growth Regulation,* in press, 1989.

Carter, J.W., Gerrard, J.M., Ready, A.E., Singhroy, S., and **Puta, E.** The effect of exercise on bleeding time and local production of prostacyclin and thromboxane. *European Journal of Applied Physiology,* **59:**355-359, 1989.

Chow, D.A., and **Bennet, R.D.** Low natural antibody and low in vivo tumor resistance, in xid-bearing B-cell deficient mice. *J. Immunol.*, **142:**3702-3706, 1989.

Dyck, D.G., Osachuk, T.A.G., Nemeth, R., Driedger, S.M. and **Greenberg, A.H.** Drug-induced (poly I:C) pyrexic responses: Congruence with a compensatory conditioning analysis. *Psychobiology,* **17:**171-178, 1989.

Gerrard, J.M. Measurements of 6-keto prostaglandin $F_{1\alpha}$ and thromboxane B_2 in bleeding time blood relation to bleeding and vascular disorders. *Can. J. Physiol. Pharmacol.* **67:**922-928, 1989.

Gerrard, J.M., Taback, S., Singhroy, S., Docherty, J.L., Kostolansky, I., McNichol, A., Kobrinsky, N.L., McKenzie, J.K., Rowe, R. In vivo measurement of thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ in humans in response to a standardized vascular injury and the influence of aspirin. *Circulation* **79:**29-38, 1989.

Gerrard, J.M., Beattie, L.L., Park, J., Israels, S.J., McNicol, A., Lint, D., Cragoe, E.J. Jr. A role for protein kinase C in the membrane fusion necessary for granule secretion. *Blood.* **74:** 2406-2413, 1989.

Gerrard, J.M., Robinson, P. Identification of the molecular species of lysophosphatidic acid produced in thrombin-stimulated platelets. *Biochimica et Biophysica Acta,* **1001:**282-285, 1989.

Gerrard, J.M., Docherty, J.C., Israels, S.J., Cheang, M.S., Bishop, A.J., Kobrinsky, N.L., Schroeder, M.L., Israels, E.D. A reassessment of the bleeding time: Association of age, hematocrit, platele function, von Willebrand factor and bleeding time thromboxane B2 with the length of the bleeding time. *Clin. Invest. Med.*, **12:**165-171, 1989

Gerrard, J.M., McNicol, A., Klassen, D., Israels, S.J. Protein Phosphorylation and its relation to platele function. In: *Blood Cells and Arteries in Hypertension and Atherosclerosis,* P. Meyer and P. Marche (Eds.) Raven Press, New York, pp 93-114, 1989.

Gerrard JM, Saxena SP, Becker AB, LaBella FS and Brandes LJ. Histamine synthesis and function in activated platelets; similarity of activation messages involved in signal transduction in platelets to these found during cell proliferation. *Thrombosis and Hemostasis* **62:**1580, 1989.

Gerrard, J.M., McNicol, A. Klassen, D. and **Israels, S.J.** Protein phosphry lation and its relation to platele function. In: *Blood Cells and Arteries in Hypertension and Atherosclerosis* (P. Meyer and P. Marche, Eds.) Raven Press, New York, pp. 93-114, 1989.

Gerrard, J.M., Docherty, J.C., Israels, S.J., Cheang, M.S., Bishop, A.J., Kobrinsky, N.L., Schroeder, M.L. and **Israel, E.D.** A reassessment of the bleeding time: Association of age, hematocrit, platelet function, von Willebrand factor and bleeding time thromboxane B2 with the length of the bleeding time. *Clin. Invest. Med.* **12:**165, 1989.

Gerrard, J.M., Beattie, L.L., Park, J., Israels, S.J., McNicol, A., Lint, D., Gragse, E.J. Jr. A role of protein kinase C in the membrane fusion necessary for platelet granule secretion. *Blood* **74:**2405, 1989.

Greenberg, A.H. The role of natural killer cells in inflammation and antibacterial activity. In: *Functions of the Natural Immune System*, (C.W. Reynolds and R.H. Wiltrout, Eds), Planum, N.Y., pp. 185-204, 1989.

Khalil, N. and **Greenberg, A.H.** Natural killer cell regulation of murine embryonic pulmonary fibroblast survival in vivo. *Cell Immunol.*, **120:**439-449, 1989.

Khalil, N., Bereznay, O., Sporn, M. and **Greenberg, A.H.** Macrophage production of transforming growth factor-ß (TGF-ß) and fibroblast collagen synthesis in chronic pulmonary inflammation. *J. Exp. Med.* **170:**727-737, 1989.

McDonald, B.E., Gerrard, J.M., Bruce, V.N., Corner, E. Comparison of the effect of canola oil and sunflower oil on plasma lipids and lipo-proteins and on in vivo thromboxane A2 and prostacyclin production in healthy young men. *Amer. J. Clin. Nutr.* **50:**1382-1388, 1989.

McNicol, A., Saxena, S.P., Brandes, L.J., Gerrard, J.M. A role for intracellular in ultrastructural changes induced in platelets by phorbol esters. *Arteriosclerosis.* **9:**684-689, 1989.

McNicol, A., Gerrard, J.M., MacIntyre, D.E. Evidence for two mechanisms of thrombin-induced platelet activation: one proteolytic, one receptor-mediated. Biochem. Cell Biol. 67:332-336, 1989.

Miller, V., Chow, D.A., and **Greenberg, A.H.** Natural antibodies In: *Natural Immunity*. Ed. D.S. Nelson, Academic Press, Australia, pp 172-217, 1989.

Pizzi, J.N., Gerrard, J.M. Expert system approach to assessments of bleeding predisposition in tonsillectomy/adenoidectomy patients. *International Journal of Pattern Recognition and Artificial Intelligence.* In press, 1990.

Roussel, E. and **Greenberg, A.H.** Identification of a macrophage activating factor (MAF) in the granules of the RNK large granular lymphocyte (LCL) leukemia. *J. Immunol.* **142:**543-549, 1989.

Saxena, S., Brandes, L.J., Becker, A. Simons, K., Gerrard, J.M. Histamine is an intracellular messenger promoting platelet aggregation. *Science* **243:**1596-1599, 1989.

Saxena, S.P., McNicol, A., Brandes, L.J., Becker, A.B., and **Gerrard, J.M.** Histamine formed in stimulated human platelets is cytoplasmic. Biochem. and Biophys. Res. 164: 164-168, 1989

Saxena SP, McNicol A, Brandes LJ, Becker AB, Gerrard JM. Intracellular histamine synthesis is critical for collagen induced platelet aggrega tion. Thrombosis and Hemostasis 62:1054, 1989.

PUBLISHED ABSTRACTS

Brown, R., Li, Z., Vriend, C., Janz, L., Falk, J., Dyck, D.G. and **Greenberg, A.H.** Evidence for an Immunosuppressive signal originating in the central nervous system. New York Academy of Sciences Meeting, Neuroimmunomodulation. New York, N.Y., 1989.

Chow, D.A., and **Tough, D.F.** Nab recognition of H-ras transformed 10T 1/2 cells. J. Cellular Biochemistry Suppl. 13B, 47, 1989.

Chow, D.A. RNK cytotoxic granule extract: Differential sensitivity of an NK-sensitive and NK-resistant murine lymphoma. *Nat. Imm. and Cell Growth Regu.* 132, 1989.

Chow, D.A. RNK cytotoxic granule extract: Differential sensitivity of an NK-sensitive and an NK-resistant murine lymphoma. 7th Int. Congress of Immunol., Berlin, Abstracts, 337, 1989.

Chow, D.A. Natural anti-tumor antibodies: Origin, genetics, specificity and in vivo activity. Society for Biological Therapy, Immunity to Cancer, Williamsburg, Virginia, Abstracts, 6, 1989.

Chow, D.A. RNK cytotoxic granule extract: Differential sensitivity of an NK-sensitive and an NK-resistant murine lymphoma. *Proceedings of the VI Int. NK Workshop*, Goslar, West Germany, 1989, in press.

Chow, D.A., and **Reese, M.R.** Tumor progression in vivo: Increased SBA lectin binding and liver metastasis. *J. Cell. Biochem. Suppl.* **14A:**187, 1990.

Gerrard, J.M., Saxena, S.P., Becker, A.B., LaBella, F.S. and **Brandes, L.J.** Histamine synthesis and function in activated platelets; similarity to activation messages involved in signal transduction in platelets to these ound during cell proliferation. *Thrombosis and Hemostasis*, **62:**1580, 1989.

Gerrard, J.M., Lint, D., Israels, S.J., Sims, P.J., Wiedmer T., Fugate, D. Identification of a 40 Kd dense granule membrane protein which is deficient in a patient with the Hermansky-Pudlak Syndrome. *Blood* **74:**31a, 1989.

Houston DS, Gerrard JM Endothelium — derived relaxing factor (EDRF) inhibits intraluminal platelet aggregation, but is blocked by red blood cell (RBC) haemoglobin (Hb). *FASEB Journal* **3:**A1179, 1989.

Israels, S.J., Daniels, M. Neonatal platelet function: Evaluation of dense granule secretion. *Pediatr. Res.* **25:**152A, 1989.

Israels, S.J., Kobrinsky, N.L. Serious reactions to desmopressin in achild with cyanotic heart disease. *N. Engl. J. Med.* **320:**1563, 1989 (L).

Khalil, N., Bereznay, O., Spor M., Greenberg, A.H. Macrophage productionof transforming growth factor-ß (TGF-ß) and fibroblast collagen synthesis in chronic pulmonary inflammation. New York Academy of Sciences Meeting, Transforming Growth Factor - ßs, Chemistry, Biology and Therapeutics, Washington, D.C., 1989.

McNicol A., Saxena S.P., Brandes L.J., Gerrard J.M. Histamine produced intracellularly in response to collagen acts to promote aggregation at a step independent of, or distal to, phosphoinositide metabolism and increased cytosolic calcium. *Thrombosis and Hemostasis* **62:**1580, 1989.

McNicol A., Saxena S.P., Brandes L.J., Gerrard J.M. Evidence that histamine has a major role in collagen but no in thromboxane activation of platelets. *FASEB Journal* **3:**A309, 1989.

Rao, G.H.R., Gerrard, J.M., Cohen, I., Witkop, C.J. Jr. and **White, J.G.** Origin and role of calcium in platelet activation-contraction- secretion coupling. In: *Cell Calcium Metabolism*, Fiskum, G. (Ed.) Plenum Publishing Corporation, pp. 411-425, 1989.

Roussel, E. and **Greenberg, A.H.** Human $CD3^-$ LAK cell granules contain a macrophage activating factor that induces macrophage (MPH) H_2O_2 production and tumoricidal activity but not Ia antigen expression. Canadian Society for Immunology Meeting, Mont St. Gabriel, Quebec, 1989.

Roussel, E. and **Greenberg, A.H.** NK-MAF: A heat stable peptide contained in large granular lymphocyte (LCL) granules that activates macrophages. *Natural Killer Cells*. (E.W. Ades and C. Lopez, Eds) S. Karger, Basel, pp.146-150, 1989.

Roussel, E. and **Greenberg, A.H.** Human LAK cell granules contain a macrophage activating factor that activate macrophage H_2O_2 and not tumoricidal activity. Seventh International Congress of Immunology, Berlin, 1989.

Saxena SP, McNicol A, Brandes LJ, Becker AB, Gerrard JM. A role for intracellular histamine in collage-induced platelet responses. *FASEB Journal* **3:**A307, 1989.

Shi, L., Battistuzzi, S., Kraut, R. and **Greenberg, A.H.** Identification and partial purification of a nucleolytic factor (NF) in granulesof the RNK large granular lymphocyte leukemia. Canadian Society for Immunology Meeting, Mont St. Gabriel, Quebec, 1989.

Tough, D.F. and **Chow, D.A.** NAb recognition of H-ras transformed 10T1/2 cells. 7th Int. Congress of Immunol., Berlin, Abstracts, 337, 1989.

EPIDEMIOLOGY AND BIOSTATISTICS

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AWARDS AND HONOURS

Nung Won Choi

Chairman: Non-Communicable Disease Section, International Conference on Epidemiology, Beijing, April 1989.

Consultant: U.S. NCI SEER Programme Evaluations, Bethesda, Maryland, June 1989 and July 1989.

Consultant: Western Pacific Regional Office of the World Health Organization on Non-Communicable Disease Control, Manila Philippines, November 1989 and March 1990.

POSTDOCTORAL AWARDS

Hee Choul Oh, Yonsei University, Korea
Manitoba Cancer Foundation Fellowship Award

Pei-Zhong Wang, Tianjin Medical College, People's Republic of China
University of Manitoba/Health and Welfare Canada

REPORT OF THE DIRECTOR

Nung Won Choi, M.D., Ph.D.

We are very pleased to report another fruitful year of research activities. Some of the studies reported last year as ongoing ones have been successfully completed and will be reflected in the list of future publications. In the current report, only those that are still continuing and those that are newly introduced are listed.

These research projects would not have been possible without the financial support and/or collaboration of the Manitoba Cancer Treatment and Research Foundation, the Manitoba Medical Services Foundation, governments of the Province of Manitoba and the City of Winnipeg, Health and Welfare Canada, particularly its Bureau of Radiation and Medical Devices, the Environmental Health Directorate and Medical Services Bureau, the Epidemiology Unit of the National Cancer Institute, many Canadian universities and cancer research centres, and the International Agency for Research on Cancer, Lyon, France.

In the following, the progress of the projects is briefly summarized.

CASE-CONTROL STUDY OF LUNG CANCER AND EXPOSURE TO RADON AND RADON DAUGHTERS

Phase I

N. W. Choi, N. A. Nelson, P. F. Moodie, J. Du, C. Sobie, C. Strutt, J. Stasiuk, M-P Regan, H. C. Oh, P-Z Wang

Much has been studied and discussed about occupational and indoor air pollution over many decades as risk factors of cancer. Only in this decade epidemiologists began to pay attention to the importance of air pollution from coal burning for heating and cooking, involuntary smoking, naturally occurring agents, as risk factors of cancer.

In North America and Northern Europe, epidemiologists have recognized the importance of radon gas as a potential agent for risk (added/interactive) of lung cancer.

Winnipeg (Manitoba) has been carrying out a case-control study to assess risks carried by high radon levels for lung cancer. Winnipeg is one of the areas in Canada where the highest levels of radon are recorded. For the past five years the department of Epidemiology and Biostatistics has been working on a study involving a total of 750 lung cancer cases as well as 750 controls, using a standard questionnaire and placing dosimeters in subjects' homes to test radon levels. This phase of the study will be completed by June 1990. All questionnaires will be input and data on radon levels will be analyzed in Ottawa by the Bureau of Radiation and Medical Devices, Health and Welfare Canada.

Phase II

N. W. Choi, N. A. Nelson, J. Du, J. Stasiuk, S. Cormack, L. Archer, S. Elgert, J. Wolfe, A. Glidden, J. Redden, M-P Regan

In 1989 this phase of the study was started whereby we endeavour to test radon levels in the subjects' previous residences. We are testing previous Winnipeg residences only. This phase was designed to obtain lifetime exposure to radon. An estimated 3.8 previous homes for each subject are being treated. After thoroughly tracing residences mentioned in the main questionnaire, researchers are asking present occupants to participate in the study. By the end of this phase, 7200 homes will have been traced

and tested for radon. All data relating to previous homes will have been computerized.

SURVEILLANCE OF ENVIRONMENTAL ASPECTS RELATED TO CANCER IN HUMANS (SEARCH)

SEARCH is a programme of the International Agency for Research on Cancer (IARC), whose principal objective is to generate, formulate and test by epidemiological methods, and on an international basis, hypotheses relating to putative risk factors for cancer occurrence.

The studies underway have attracted considerable scientific interest and have been funded in centres scattered throughout the world, thus bringing together leading cancer epidemiologists to tackle the same problem.

CHILDHOOD BRAIN CANCER CASE-CONTROL STUDY

N. W. Choi, N. A. Nelson, P. F. Moodie, J. Ediger

Brain tumours in children are sufficiently uncommon as to make their study in any single centre exceedingly difficult. Under the SEARCH umbrella, Winnipeg, Paris, Milan, New South Wales, Valencia and Los Angeles are participating in this study. What little is known about the etiology of this form of cancer has been incorporated into a study where the main hypothesis has been recently suggested by laboratory findings: the role of nitrosamines, nitrosatable substances and inhibitors of nitrosation on the risk of this disease. This includes sources such as nitrosamine exposure from passive smoking and from dietary sources as well as intake of nitrate and nitrite from food and water with information also collected about vitamin C intake, which has been demonstrated in man as being able to inhibit nitrosamine formation from amino substrates.

Manitoba's involvement in this study has produced 31 cases and 62 controls, and data will shortly be sent to IARC in Lyon, France, to be pooled with the data from other centres and then analyzed.

ADULT BRAIN CANCER CASE-CONTROL STUDY

N. W. Choi, N. A. Nelson, P. F. Moodie, J. Ediger

As in the Childhood Brain Study, this is also an international collaborative project. This strength of this study, apart from the level of interest of collaborators from Winnipeg, Stockholm, Vilnius, Toronto, Adelaide, Milan, Athens and Heidelberg, lies in the reasonable number of focussed hypotheses which require a questionnaire needing approximately one hour to administer to cases and controls alike.

Manitoba's contribution to the data base is 164 cases and 325 controls. Data will be sent to IARC, Lyon, France, shortly to be pooled with data from the other centres, then analyzed.

CASE-CONTROL STUDY OF PANCREATIC CANCER

N. W. Choi, N. A. Nelson, P. F. Moodie, M-P Regan

This is the first of three studies in the SEARCH Programme in which our department has been involved over the years. This also was a case-control study initiated in 1983 with a series of pilot studies designed to ascertain the feasibility of obtaining data relating to lifestyle factors in the etiology of pancreatic cancer. Manitoba's contribution to the data base is 100 cases and 100 controls.

Data collection has been finalized in all six collaborating centres (Toronto, Winnipeg, Montreal, Warsaw, Utrecht and Adelaide), and the final data base has been assembled in Lyon. Results of this study will be presented in the IARC Scientific Publications Series.

CANCER AMONG REGISTERED INDIANS IN MANITOBA

N. W. Choi, N. A. Nelson, P. F. Moodie, P-Z Wang, H. C. Oh, R. M. Musto

The first phase of this study was to identify those Indians who have been diagnosed with cancer during the nine year period 1978-1986. This involved record linkage of Medical Services Bureau data base to that of the Manitoba Cancer Registry. The Medical Services Bureau data base comprises 66,219 registered Indians; that of the Cancer Registry, 63,061 cases. An identifier common to the two data bases is the Manitoba Health Services Commission number. However, as this number does not uniquely identify an individual, multistage matching using additional identifiers is being employed. Twenty-one percent of the individuals in the Manitoba Indian Registry data base did not have a valid M.H.S.C. number recorded. Thus an alternate multistage linkage pathway is being developed for them.

Preliminary analyses indicate that while many cancer sites are found to be in deficit, cancers such as cancer of the buccal cavity and cancers of the cervix and urinary organ are in excess for males and females respectively. For males, the relative risk of cancer comparing the observed number of cases in the Indian population with the expected number based on total Manitoba rates was 0.372 ($p < .001$, 2-tailed Poisson). This deficit varied from site to site ($X^2(19) = 173.59$ giving statistically significant interaction by site, $p < .001$). Cancers of the buccal cavity (ICD 140–149) were in excess for the Indian population (relative risk = 1.55, $p < .02$). Great deficits were observed in the numbers of skin (ICD 173) and prostate (ICD 185) cancers ($P < .001$ for both sites). Among females the 'all site' relative risk was 0.552 ($p < .001$, 2-tailed Poisson). Once again a statistically significant variation in relative risks between sites was found ($P < .001$). Cervical cancers (ICD 180) were in excess (relative risk = 2.221, $p < .001$). Kidney and other urinary cancers omitting bladder cancers were in excess among women age 30–74 (relative risk = 2.32, $p < .001$). Considerable deficits were found for colon and rectal cancers (ICD 153-154), cancers of bone, connective tissue and melanoma (ICD 170-172), skin cancers (ICD 173), breast cancers (ICD 174) and uterine cancers (ICD 182).

The second phase of the study will involve further investigation of certain cancer sites found to be statistically significantly in excess or in deficit by means of a case-control study. A standardized questionnaire will be used requiring the assistance of nursing station personnel.

CASE-CONTROL STUDY OF PESTICIDE USE AND HEALTH

N. W. Choi, H. H. McDuffie, J. Ediger

In 1989, preparations got underway for the start of a case-control study of pesticide use and health.

This study involves six Canadian centres who will look into the recent evidence suggesting that the use of agricultural herbicides, in particular, 2,4-D has been associated with an increased risk for farmers to develop non-Hodgkin's lymphoma. A group of investigators from six Canadian centres will be carrying out a case-control study of soft tissue sarcoma, Hodgkin's disease, and non- Hodgkin's lymphoma, with special emphasis on pesticide exposure.

This study will utilize a mail-in questionnaire to obtain information about pesticide exposure, demographics, geographical locations, occupations, and some behavioural factors in cancer cases and controls.

CHILDHOOD LEUKEMIA STUDY AND LOW FREQUENCY ELECTROMAGNETIC FIELD EXPOSURE

N. W. Choi, J. Gerrard, M. McBride, J. Ediger, K. Hjalmarsson

In 1989, background work has been done. Leukemia is the most common childhood malignancy in Canada. Several recent studies have suggested that children exposed to strong low frequency electrical or magnetic fields may be at high risk of leukemia.

This is a multi-centre study designed to obtain sufficient leukemia cases to adequately measure any relationship among different types of leukemia.

We propose to conduct a case-control study on the possible association between exposure to electrical and magnetic fields, and subsequent risk of leukemia in children age 0-14.

Children diagnosed with leukemia over a five year period (1990–1994), residing in one of the principal cities and surrounding areas of each of the participating provinces, will be eligible for inclusion in the study if their disease is in remission. A standard questionnaire will be utilized and each child will be asked to wear a small electromagnetic field dosimeter for a period of 48 hours during which EMF exposure will be recorded.

BIOSTATISTICS RESEARCH ACTIVITIES

N. A. Nelson and P. F. Moodie

In addition to providing consultation and statistical analyses for other departments in the Foundation, two of the section's major focuses have been computerized record linkage and meta-analysis.

For the in-house project on Cancer Among Registered Indians in Manitoba we have developed on the Manitoba Cancer Treatment and Research Foundation Prime mini-computer methodologies and software which link two unrelated large registration data bases, that of the Manitoba Registered Indians and that of the Foundation's cancer registry. Most other studies on record linkage to date have required large main frame computers: thus this work serves as a feasibility study of linkage implemented on a more restricted computer resource.

Meta-analysis is the analysis applied to quantitative summaries of individual research studies. In the field of clinical trials and epidemiology, the need is great for meta-analysis as many individual clinical trials and cancer epidemiologic studies are too small to obtain conclusive results. In addition, there may be considerable variation in results from one study to another. Collaborative studies with a number of centres participating probably give the most definitive results. However, hypotheses which motivate such large studies may come from a number of smaller studies which should be summarized. The summaries with thorough analyses can in themselves be conclusive if one does not select studies based on their results.

A selection bias can occur if studies which do not find statistically significant associations between risk factors and disease or studies which do not find statistically significant differences in treatment effects tend to not be published.

It can also occur if such studies are reported but too little information is given to be included in the summary. When this is the case a disproportionate number of studies with positive findings are included in the summary.

Our section's current interest is in investigating and developing meta-analysis procedures which provide overviews of clinical trials, in particular where survival is one of the outcomes of interest.

PUBLICATIONS

Boice, J. D., Blettner, M., Choi, N. W. et al. Radiation dose and breast cancer risk in patients treated for cancer of the cervix. Int. J. Cancer **44**:7-16, 1989.

Shore, T., Nelson, N. A., Weinerman, B. A Meta-Analysis of Stages I and II Hodgkin's Disease. Cancer **65**:1155-1160, 1989.

Kaldor, J. M., Day, N. E., Choi, N. W. and IARC Working Group. Leukemia Following Chemotherapy for Ovarian Cancer. N. Engl. J. Med. (in press), 1989.

Kaldor, J. M., Day, N. E., Choi, N. W. and IARC Working Group. Leukemia Following Hodgkin's Disease. N. Engl. J. Med. (in press), 1989.

MEDICAL PHYSICS

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AWARDS AND HONOURS

JEFF BEWS, Ph.D.

appointed as ADJUNCT PROFESSOR in Physics at the University of Manitoba.

appointed to the SCIENTIFIC STAFF of the Department of Radiology at the Health Sciences Centre.

WALTER HUDA, Ph.D.

appointed VICE-PRESIDENT of the Canadian College of Physicists in Medicine.

appointed to the BIOMEDICAL ENGINEERING PANEL of the Canadian Medical Research Council (MRC).

appointed to the ATOMIC ENERGY CONTROL BOARD (AECB) Working Group on the new ICRP radiation protection recommendations.

certified by the AMERICAN BOARD OF MEDICAL PHYSICS (ABMP) in Diagnostic Imaging Physics.

promoted to ASSOCIATE PROFESSOR in Radiology (U of M)

SHLOMO SHALEV, Ph.D.

was an INVITED SPEAKER in the Radiation Oncology Visiting Professor Series, University of Michigan, Ann Arbor.

was an INVITED SPEAKER at the University of Rochester Cancer Center, Rochester N.Y.

was an INVITED SPEAKER at the University of Chicago Medical Center.

was an INVITED SPEAKER at the University of North Carolina, Chapel Hill.

was a SYMPOSIUM SPEAKER at ONCOVAIL, Vail Colorado.

was a SYPMOSIUM SPEAKER at the AAPM Spring Seminar, Las Vegas.

POST-DOCTORAL AND STUDENT AWARDS:

KONSTANTINOS CHANTZIANTONIOU, B.Sc.

received a Manitoba Cancer Treatment and Research Foundation STUDENTSHIP.

TERRY CHU, B.Sc.

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received a National Science and Engineering Research Council STUDENTSHIP.

KONRAD LESZCZYNSKI, M.Sc.

received a Manitoba Health Research Council STUDENTSHIP and received a STEVE FONYO STUDENTSHIP from the National Cancer Institute of Canada.

JOHN MCLELLAN, B.Sc.

received a University of Manitoba GRADUATE FELLOWSHIP.

VISITING SEMINAR SPEAKERS

The Chernobyl Accident and Its Consequences

J. Jovanovich, Ph.D., Department of Physics, University of Manitoba

Cold Fusion Confusion

Kumar Sharma, Ph.D., Department of Physics, University of Manitoba

Manitoba: A Warmer and Drier Tomorrow?

Hugh Fraser, M.A.,

Physics of Radiosurgery

Erwin Podgorsak, Ph.D., Montreal General Hospital

ON-LINE PORTAL IMAGING

S. Shalev, K.W. Leszczynski, S. Cosby

A video imaging system has been developed for on-line verification of radiation treatment accuracy. The detector consists of a metal plate with a fluorescent screen, which is viewed through a 45° mirror by a sensitive video camera. Image acquisition and processing are performed with an Imaging Technology Series 151 processing system and a PC-AT host. Both static and dynamic (movie) images are obtained of the patient during treatment. Spatial resolution and signal-to-noise were optimized to provide high quality images under clinical conditions. Patients undergoing treatment in the head and neck or breast were studied, and both inter-treatment organ movement and intra-treatment set-up displacements were studied.

IMAGE PROCESSING FOR ON-LINE PORTAL VERIFICATION

S. Shalev, K.W. Leszczynski

Portal images exhibit very poor contrast, and digital image processing is necessary to permit the visualization of anatomical features. We have implemented a wide range of contrast enhancement routines and compared them with conventional techniques for improving the quality of on-line portal images. An adaptive filtering method was shown to improve contrast without compromising spatial resolution. A new algorithm called moving histogram equalization (MHE) was shown to be as

Dr. S. Shalev

Mr. K. W. Leszcynski

fast and as effective as conventional methods, while reducing the artifacts usually generated in algorithms using bilinear interpolations.

AUTOMATED VERIFICATION OF TREATMENT PORTALS

S. Sbalov, K.W. Leszczynski

The success of radiation therapy depends upon the accuracy with which the prescribed radiation fields are reproduced in the course of consecutive treatment fractions. Traditionally, verification of radiotherapy treatment involves visual analysis of portal films, and is difficult and time consuming. With the advent of on-line portal imaging, automated analysis of images is required. We have investigated a number of algorithms for the detection of field edges, and have shown that the Gaussian derivative operator is superior to standard gradient techniques. It is designed to optimally match the characteristics of radiation field edges and is shown to maximize both its response to field edges relative to other edges and the accuracy of edge localization. At the same time, the number of false edge points due to the noise is minimized. The extracted field boundaries are superimposed on the original portal image to define the region over which digital enhancement techniques are applied and to allow quick portal verification by the therapist. This edge detection scheme can also be viewed as a step toward fully automated treatment verification, where the portal field contour must be compared to the prescribed portal, and any significant deviations identified very rapidly.

COMPUTERIZED ANALYSIS OF PORTAL FILMS

S. Shalev, T. Chu, S. Cosby

We have developed a simple and inexpensive system known as PEST (Portal Enhancement STation) to digitize portal films with a video camera and display the digital image in a 512x480 pixel 8-bit frame buffer. The images are enhanced by high-pass filtering and adaptive histogram equalization, and compared with a digitized simulator film by aligning selected anatomical landmarks to determine the 2-D transformation parameters corresponding to translation, rotation and scaling. The portal field edges are then transformed and overlaid on the simulator image, showing any discrepancies between the prescribed and the actual borders as regions of excessive dose (red) or underdose (blue). Measurements were carried out on 55 film pairs from 11 patients receiving radiation treatment for carcinoma of the head and neck. The average field edge error was found to be 4.2+1.3 mm, and the average displacement of the field center was 3.1+1.7 mm.

THE OPIUM PROJECT

S. Shalev, S. Cosby

A digital computer software package known as OPIUM (On-line Portal Imaging University of Manitoba) was developed and licensed to Siemens Medical Laboratories. Further development is being carried out in a collaborative project aimed at improving the speed and versatility of the package. The main design criteria are fast, automatic acquisition of on-line portal images during radiation therapy, and an easy-to-use facility to review static and dynamic portal images for each patient. As part of the collaboration, the Manitoba Cancer Foundation will act as a beta test site for the on-line portal imaging device, and will develop improved algorithms for image acquisition, correction, enhancement and display.

AN OBJECTIVE METHOD FOR ASSESSING DOSE VOLUME HISTOGRAMS

D.A. Viggars, S. Shalev

Dose-volume histograms (DVH) are a convenient means of summarizing a three-dimensional dose distribution. In order to facilitate their use in clinical decision making, an objective and quantitative technique of interpreting DVHs has been developed. The technique quantifies the deviation of the DVH for an actual dose distribution from a well-defined ideal DVH. The technique also makes use of a set of dose-volume limits for the target and sensitive organs prescribed by a radiation oncologist for a particular patient or group of patients. This dose-volume prescription is used to place tolerance limits on the deviations of the actual DVH from the ideal. A set of score functions is calculated which shows how well the dose distribution satisfies the prescribed dose-volume limits. In addition to this quantitative technique a visual means of comparison of a DVH with a dose-volume prescription, known as a 'histogram of regret', has been developed. A histogram of regret is formed by shading areas of a DVH with colours which indicate deviations from the ideal DVH. The effectiveness of these techniques has been demonstrated for cases of carcinoma of the lung and prostate.

EVALUATION OF THE CLINICAL SIGNIFICANCE OF PATIENT MOVEMENT AND SET-UP ERRORS DURING EXTERNAL BEAM RADIOTHERAPY

D.A. Viggars, S. Shalev, B. Hintz

The position of a patient during external beam radiotherapy is uncertain because of imprecision and inaccuracy in the setting up of the patient and because the patient is not a static, rigid body. In a preliminary investigation of the clinical

Dr. D. A. Viggars

significance of these effects, three dimensional dose distributions for several cases of ca prostate were calculated using rectangular and conformal four-field treatment techniques. OSCAR scores, images of regret and dose-volume histograms were calculated in each case. The effect on these characteristics of the dose distributions of uncertainty in the position of the patient was determined by recalculating the three dimensional dose distributions with the isocentre shifted by up to 0.5 cm in the lateral, A-P and P-A directions. The scores, images of regret and dose volume histograms were determined for the shifted distributions and compared with those for the unshifted distributions. The preliminary results indicated that the relatively small movements of the patient which were investigated can cause clinically significant changes in the dose distributions. However these changes are complex and require further investigation. In particular, contrary to expectation, it cannot be assumed that conformal plans are always more sensitive to movement than conventional plans using rectangular beams.

QUANTITATIVE COMPARISON OF 3-DIMENSIONAL RADIOTHERAPY TREATMENT PLANS

S. Shalev, D.A. Viggars, B. Hintz, P. Therrien

Three-dimensional dose distributions for external beam radiation treatment of ca prostate were calculated for 10 different treatment modalities: rectangular and conformal beams using 3 field, 4 field and double arc with 23 MV photons, and 5 field and double arc with Co^{60} photons. The resulting dose distributions were compared using images of regret, dose volume histograms, OSCAR scores, and risk analysis. Although tissue-specific dose

response data are sparse, it was shown that risk analysis and score functions give similar results on a relative scale, for grading alternative plans. For pelvic treatments the high energy conformal technique minimizes normal tissue complications, while the double arc technique still used in some centers was found to be quite unsatisfactory. Improvements were made in the design of the dose prescription to enhance the ease of use and acceptability by practising radiation oncologists.

THE OSCAR PROGRAM

D.A. Viggars, S. Shalev

The development of the OSCAR computer software package continued in collaboration with Theratronics International. An interactive prescription utility was written which allows radiation oncologists to create a library of dose volume prescriptions. The system is user friendly and performs checks for consistency and "reasonableness" of the prescriptions to reduce the likelihood of errors in data entry. An oncologist is able to select any prescription from the library for use with a particular patient. Software to implement histograms of regret was written, and histograms of regret can now be displayed by means of a single keystroke as soon as the dose-volume histograms have been calculated. All the new software was designed to run in the Theraplan environment, and has been incorporated by Theratronics International Ltd. into the latest release of their Theraplan system.

THE SENSITIVITY OF THE THORACO-LUMBAR SPINAL CORD OF THE MOUSE TO HYPERTHERMIA

G. Froese, R.M. Das, P.B. Dunscombe

As the techniques of hyperthermia increase in sophistication and its employment as a routine adjunctive modality in the treatment of malignancies becomes more likely, it is essential to investigate the effect of hyperthermia on those organs which frequently limit the radiation dose to the tumour. The spinal cord is one of these organs. Our study of the effect of radiofrequency-heating on the thoraco-lumbar spinal cord of mice has led to the following conclusions. The spinal cord responds acutely to hyperthermia in contrast with its late response to X- irradiation. The sensitivity in terms of developing severe neurological symptoms is greater than has been preciously expected, and the temperature-time relationship for an isoeffect and the development of thermotolerance 24 hours after a short heating time at 45°C are similar to the general finding in other tissues and tumours.

ORGAN IMAGING RESEARCH REPORTS

THE MEASUREMENT OF REGIONAL CEREBRAL BLOOD FLOW USING XENON- ENHANCED COMPUTED TOMOGRAPHY (Xe/CT)

J. Beus, P. Dunscombe, K. St.Laurence

Local cerebral blood flow and tissue partition coefficient can be measured indirectly by monitoring the temporal uptake (t=time) of xenon in brain tissue, C(t), and arterial blood, CA(t), during patient inhalation of this stable inert gas. By fitting the Kety-Schmidt equation to these data, flow and partition coefficients can be calculated. In the Xe/CT technique, C(t) is determined using dynamic computed tomography scanning, while CA(t) is deduced from thermoconductivity meter measurements of the xenon concentration in the patient's expired air. To date, our research efforts have concentrated on quantifying the precision and accuracy of clinical Xe/CT blood-flow measurements. Phantom studies have revealed that system calibration errors lead to very small inaccuracies in results, typically less than 3%. Monte Carlo computer simulations, meanwhile, have indicated that CT scanner noise is a significant source of error and in fact puts an upper limit on the resolution (localization) attainable with this technique. Computer simulations also have shown that the precision and accuracy associated with Xe/CT blood flow measurements are inferior to that of Positron Emission Tomography (PET) based techniques for all but the smallest tissue regions.

ELECTRON DOSIMETRY

G.A. Sandison, W. Huda, L. Papiez J. McLellan, P. Therrien

This research project was funded by the National Cancer Institute of Canada, and is aimed at improving the computation of dose distributions associated with electron beams. Significant progress has been made in the development of new theoretical models for electron beams (e.g. electron loss model), and in producing a computer algorithm for generating dose (fluence) distributions superimposed on to CT data obtained on the HSC CT scanners.

BIOMEDICAL NUCLEAR MAGNETIC RESONANCE

J. Peeling, W. Huda, G. Froese, J. Beus

A new 7 Tesla (300 MHz) NMR spectrometer was obtained in collaboration with the Canadian National Research Council (NRC). Research activities which are currently underway include the development of new magneto-pharmacueticals, and the study of anoxic cells during radiotherapy.

REGIONAL CEREBRAL BLOOD FLOW

M. Mirzai, R. Palser

A computer simulation of the measurement of regional cerebral blood flow from single photon emission computed tomographic images is under development. A four-compartmental model and the radiopharmaceutical Tc-99m-HMPAO are being used together with typical nuclear medicine scan data and arterial blood activity concentration curves to determine the blood flow to regions of the brain under typical clinical conditions.

MEDICAL DOSIMETRY

W. Huda, A.M. Sourlies, J. Beus

Work has continued on developing improved techniques for assessing the radiation doses (and corresponding risks) to patients undergoing diagnostic exami-

nations which employ ionizing radiations. Specific applications include the radiation doses associated with Positron Emission Tomography (PET), mammography, diagnostic x-rays and diagnostic (nuclear medicine) thyroid examinations. In addition, a new concept (Population Irradiation Factor) has been developed to take into account patient demographics for determining the radiation detriment associated with medical uses of radiation.

CARDIAC VOLUMES

W. Huda, M. Baker, I.D. Greenberg, R. Corne

This study is designed to develop a non-invasive technique for the determination of left ventricular volumes in Nuclear Medicine Multi-gated Acquisition (MUGA) studies. The NM results are to be compared with Magnetic Resonance Cardiac studies to be performed on the St. Boniface MR unit in the summer of 1991.

AN X-RAY TUBE FOCAL SPOT SIZE MEASUREMENT TECHNIQUE USING A DIGITAL IMAGING COMPUTER METHOD

K. Chantziantoniou and A.M. Saurkes

A digital imaging computer-system method is being developed for evaluating the dimensions of a diagnostic x-ray tube focal spot. The method involves digitizing the x-ray image of a specially designed parallel wire focal spot test tool and evaluating the results using an appropriately-designed computer algorithm applied to the digitized image of the test tool image captured by a micro-computer-linked television camera using suitable frame-grabbing and digitizing computer electronics.

Dr. W. Huda

PUBLICATIONS:

Dunscombe, P.B. and **Fox, K.** Precision of determining compliance with prescribed fields form conventional portal films. *Br. Journal of Radiology*, **62**:49-52, 1989.

Dunscombe, P.B. The Teaching of Physics and Related Courses to Residents in Radiation Oncology. *Journal of the Canadian Association of Radiologists*, **40**:211-212, 1989.

Dunscombe, P.B., Cetas, T.C., Connor, W.G., Couple, E.B., Hetnel, F.W., Lee, W.K., Loshek,D., Nussbaum, G.H., Roemer, R., Saylor, T.K., Strohbehn, J.W., Waterman, F.M., Samulski, T.V., Paliwal, B.R. Hyperthermia Treatment Planning. *AAPM Report* No. 27, 1989.

Huda, W. and **Sandison, G.A.** The use of the effective dose equivalent, H_E, for ^{99m}Tc labelled radiopharmaceuticals. *Eur. J. Nucl.Med.*, **15**:174- 179, 1989.

Huda, W., Sandison, G.A., and **Lee, T.Y.** Patient doses from computed tomography in Manitoba from 1977 to 1987. *The British Journal of Radiology*, **62**:138-144, 1989.

Huda, W. and **Scringer, J.W.** Irradiation of Volunteers in Nuclear Medicine. *Journal of Nuclear Medicine*, **30**:260-264, 1989.

Huda, W., Lentle, B. and **Sutherland, J.B.** The Effective Dose Equivalent in Radiology. *J. Canadian Assn. of Radiologists*, **40**:3-4, 1989.

Huda, W. and **Gordon, K.** Nuclear Medicine Staff and Patient Doses in Manitoba (1981-1985). *Health Physics*, **56**:277-285, 1989.

Huda, W. and **Boutcher, S.** Should Nuclear Medicine Technologists Wear Lead Aprons? *J. Nucl. Med. Tech.*, **17**:6-11, 1989.

Huda, W., Bews, J. and **Sourkes, A.M.** Occupational Doses in Radiation Oncology in Manitoba - 1980 to 1986. *Health Physics*, **57**:521-527, 1989.

Huda, W., Sandison, G.A., Palser, R.F. and **Savole, D.** Radiation Doses and detriment from chest x-ray examinations. *Phys.Med.Biol.*, **34**:1477- 1492, 1989.

Huda, W., Bews, J. and **Saydak, A.P.** Radiation doses in extracorporeal shock wave lithotripsy. *Br. Journal of Radiology*, **62**:921-926, 1989.

Huda, W. and **Sourkes, A.M.** Radiation Doses from Chest X Rays in Manitoba (1979 and 1987). *Rad. Prot. Dosimetry*, **28**:305 -308, 1989.

Huda, W. and **Sourkes, A.M.** Individual and population doses in Manitoba from chiropractic X-ray procedures. *J. Radiol. Prot.*, **9**:241-245, 1989.

Huda, W., Sandison, G.A. and **Lee, T.Y.** CT usage in Manitoba (1977-1987) and the corresponding patient doses. *British Journal of Radiology*, **62**:198-204, 1989.

Huda, W. and **Scringer, J.W.** Volunteers in Nuclear Medicine clinical trials. *Journal of Nuclear Medicine*, **30**:260-264, 1989.

Huda W., Lentle, B.C. and **Sutherland, J.B.** Effective dose equivalent (Letter). *Journal of the Canadian Association of Radiologists*, **40**:552-555, 1989.

Huda, W. and **Scringer, J.W.** Irradiation of volunteers in Nuclear Medicine (Letter). *Journal of Nuclear Medicine*, **30**:2062-2065, 1989.

Huda, W. and **Sourkes, A.M.** Population doses from chest X-rays in Manitoba (1979 - 1987). *Radiation Protection Dosimetry*, **28**:305-308, 1989.

Leszczynski, K.W. and **Shalev, S.** A robust algorithm for contrast enhancement by local histogram modification. *Image and Vision Computing*, **7**:205-209, 1989.

Leszczynski, K.W. and **Shalev, S.** Digital contrast enhancement for on line portal imaging. *Medical & Biological Engineering & Computing*, **27**:507-512, 1989.

Sandison, G.A. and **Huda, W.** Is the 'fictitious' virtual source a redundant concept for scanned therapeutic electron beams? *Phys.Med.Biol.*, **16**:569-578, 1989.

Sandison, G.A., Huda, W. and **Savole, D.** Comparison of methods to determine

electron pencil beam spread in tissue-equivalent media. *Med. Phys.*, **16**:881-888, 1989.

Sandison, G.A. and **Huda, W.** Is a virtual source necessary to predict output factors for electron accelerators. *Physics in Medicine and biology*. **34**:369-378, 1989.

Sandison, G.A., Huda, W., Battista, J.J. and **Savole, D.** An investigation of three methods to determine electron pencil beam spread parameter in tissue-equivalent media. *Medical Physics*, **16**:881-888, 1989.

Shalev, S., Lee, T., Leszczynski, K., Cosby, S., Chu, T., Reinstein, L., and **Meek, A.** Video Techniques for On-Line Portal Imaging. *Computerized Medical Imaging and Graphics*, **13**:217-226, 1989.

Sutherland, J.B. and **Huda, W.** Cost-benefit analysis of non-ionic contrast agents. *Journal of the Canadian Association of Radiologists*, **40**:18- 21, 1989.

Viggars, D., Shalev, S., and **Hahn, P.** Optimization by minimizing regret. In *Prediction of Response in Radiation Therapy*. (B.R. Paliwal, J.F. Fowler, D.E. Herbert, T.J. Kinsella and C.G. Orton, eds.). *AAPM Symposium Proceedings*, **7**:289-295, 1989.

PUBLISHED ABSTRACTS

Bews, J., Huda, W., Sourkes, A.M., Radiation doses from mammographic procedures in Manitoba: 1978-1988. *Radiology Supplement* **173(P),** 394, 1989.

Chu, T., Shalev, S., Leszyznski, K. and **Cosby, S.** The digital analysis of portal films. *Medical Physics* **16**:475, 1989.

Leszczynski, K. and **Shalev, S.** Adaptive noise filtration for video portal images. *Medical Physics* **16**:496, 1989.

Leszczynski, K., Shalev, S. and **Cosby, S.** Automated delineation of field boundaries for radiation therapy treatment verification. *Radiology* **173(P),** 90, 1989.

Shalev, S., Leszczynski, K. and **Reinstein, L.** Design and evaluation of a video portal imaging system. *AAPM Spring Seminar, Las Vegas, April 1989.*

Shalev, S., Leszczynski, K., Cosby, S., Reinstein, L., and **Meek, A.** Online portal imaging: Design and evaluation of a video imaging system. *Int. Congress of Radiology, Paris, July 1989.*

Shalev, S. and **Viggars, D.** Dosimetric implications of organ movement in radiotherapy. *Int. Congress of Radiology, Paris, July 1989.*

Shalev, S., Therrien, P. and **Viggars, D.** Optimization of treatment plans by risk analysis. *Int. Congress of Radiology, Paris July 1989.*

Shalev, S., Viggars, D. and **Hahn, P.** The evaluation of 3-dimensional dose distributions using dose volume histograms. *Int. Congress of Radiology, Paris, July 1989.*

Shalev, S., Chu, T., Cosby, S. and **Leszczynski, K.** Treatment of verification by digital analysis of portal films. *Int. Congress of Radiology, Paris, July 1989.*

Shalev, S., Viggars, D., Reinstein, L., Leszczynski, K., Cosby, S. and **Chu, T.** The dosimetric implications of patient movement during radiation treatment. *Int J Rad Onc Biol Phys. 17, Sup. 1, 158, 1989.*

Shalev, S., Cosby, S., Leszczynski, K. and **Chu T.** Data base for radiation therapy images. *Radiology* **173(P),** 89, 1989.

Viggars, D., Shalev, S. and **Stewart M.** The interpretation of dose volume histograms. *Int. J. Rad. Onc. Biol. Phys.* **17,** *Sup. 1, 125, 1989.*

Viggars, D. and **Shalev, S.** Use of a concise prescription for specifying absolute dose distributions in external beam radiation therapy. *Radiology* **173(P),** *163, 1989.*

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BASIC RESEARCH

Doctors J. B. Johnston, G. J. Goldenberg, L. J. Brandes and B. A. Schacter as members of the Department of Medical Oncology are also senior members of the Scientific Staff of the Manitoba Institute of Cell Biology. Descriptive abstracts of their ongoing basic research are to be found in Cell Biology's (separate) report.

CLINICAL INVESTIGATION

New developments in medical therapy of cancer have made the conduct of a clinical investigation programme the cornerstone of developing cancer treatment programmes. In Medical Oncology, programmes of clinical investigation are carried out, both on an independent basis, and in collaboration with major clinical trial co-operative groups.

The Protocol Review Committee, with multidisciplinary membership, evaluates and approves all new clinical protocols involving the Manitoba Cancer Treatment and Research Foundation patients. Committee membership includes Drs. M. Levitt (Chairman), D. Bowman, B. Weinerman, G. Krepart, and L. G. Israels.

The Department of Medical Oncology also participates in clinical trials sponsored by several national and international clinical trial co-operative groups, including the National Cancer Institute of Canada (NCIC - Principal Investigator - Dr. M. Levitt), and the National Surgical Programme for Breast and Bowel Cancers (NSABP - Principal Investigator, Dr. D. M. Bowman). Participation in numerous protocols with these co-operative groups and institutions remains a high priority of medical oncology. We believe these multi-institutional co-operative trials represent the forefront of developmental cancer therapeutics.

The Clinical Investigation Office, directed by Dr. M. Levitt, medical oncologist, coordinates all MCTRF clinical trials, a summary of which follows:

SUMMARY OF 1989 STATISTICS

CLINICAL INVESTIGATIONS OFFICE ACCRUAL — ADULT

STUDY GROUP:		
NCIC	**1988**	**1989**
Remaining On Study From Prior Years	312	307
New patient Accrual	17	28
Off Study	22	28
Remaining On-Study	307	307
NSABP		
Remaining On Study From Prior Years	386	396
New Patient Accrual	33	29+*8= 113
Off Study	23	9+*8= 105
Remaining On Study	396	404
RTOG (New Institution — Affiliate Member as of July 1988)		
Remaining On Study From Prior Years	—	11
New Patient Accrual	11	10
Off Study	0	1
Remaining On Study	11	20
LOCAL TRIALS		
Remaining On Study From Prior Years	107	87
New Patient Accrual	12	**31
Off Study	32	**28
Remaining On Study	87	90
TOTAL ON-STUDY (YEAR END)	801	821
PEDIATRICS		
Remaining On Study From Prior Years	254	270
New Patient Accrual	37	***56
Off Study	21	13
Remaining On Study	270	313
TOTAL ON STUDY (YEAR END)	270	313

* Occupational Staff Health & Hazard Study (84 participants)
** Miscellaneous Non-protocol patients included (14 patients)
*** Includes Local and Miscellaneous (Non Protocol Patients)

1989
CLINICAL INVESTIGATIONS OFFICE NEW PATIENT ACCRUAL
ADULT

GROUP	ST. BONIFACE		M.C.F.		OTHER (VGH, GRACE, etc.)		TOTAL	
	1988	1989	1988	1989	1988	1989	1988	1989
NCIC	8	3	8	19	1	6	17	28
NSABP	14	9	10	**96	9	8	33	113
RTOG	0	0	11	10	0	0	11	10
LOCAL & OTHER	1	*21	11	*9	0	1	12	31
TOTAL	23	33	40	**134	10	15	73	182
PEDIATRICS:	CCSG/SWOG/ NWTS/LOCAL		37	*56				

*Includes miscellaneous non protocol patients
**Occupational Staff Health & Hazard Study (84)

CLINICAL INVESTIGATIONS OFFICE ADULT STATISTICS
ST. BONIFACE HOSPITAL — 1989

GROUP	ON STUDY FROM END OF		NEW PATIENTS ENTERED DURING		OFF STUDY DURING		PATIENTS ON ACTIVE FOLLOW-UP END OF	
	1987	1988	1988	1989	1988	1989	1988	1989
NCIC	72	71	8	3	9	11	71	63
NSABP	122	130	14	9	6	8	130	131
RTOG	0	0	0	0	0	0	0	0
LOCAL & OTHERS	46	29	1	*21	18	*19	29	3[1]
TOTAL	240	230	23	33	33	38	230	225

* Includes miscellaneous non protocol patients.

The following is a brief description of active adult clinical investigative trials in 1989.

Disease Site	Number	Description
Lung Carcinoma	MBR 86.1	A randomized study to compare the toxicity and effectiveness of radiotherapy plus lonidamine with "standard" radiotherapy itself in patients with inoperable non-small cell lung cancers without distant metastases.
	MBR 89.1	SCHERING — A randomized Phase III extensive small cell carcinoma lung study; in patients who have Cyclophosphamide, Adriamycin and VP-16 administered x 6 cycles, followed by Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF). Patients are randomized to various dose levels of GM-CSF: None, 10 or 20 mcg/kg.
	MBR 88.1	CARBO-CIS II — Study of Carboplatin and Cisplain in combination with etoposide in previously untreated, small cell lung cancer and in conbination with etoposide and bleomycin in previously untreated non-resectable non-small cell lung cancer.
Lymphoma	NCIC LY.3	Comparison of standard BACOP chemotherapy with escalated BACOP chemotherapy with escalaed BACOP in "poor prognosis" histology.
Leukemia	NCIC HL.1	A randomized comparison of Deoxycoformycin VS Alpha-Interferon in Previously untreated patients with Hairy Cell Leukemia.
	MLK 88.5	Resistance to alkylating agents in chronic lymphocytic leukemia.
Bone Marrow	MBM 88.5	Phase I-II Trial of Thalidomide with or without cyclosporine in the treatment of refractory chronc graft-versus-host disease (GVHD).
Hodgkins Disease	NCIC HD.4	To study the effectiveness of MOPP/ABV Hybril chemotherapy versus alternating MOPP/ABVL for remission induction, durability and survival in advanced or recurrent disease.
Multiple Myeloma	N.MY. 84.1	Pilot study of weekly Cyclophosphamide and alternate day Prednisone therapy (CWAP) as primary treatment in multiple myeloma Stage I-IV.

Malignant Melanoma	NCIC ME.5	Surgical study to determine the safest excision margins around the primary melanoma and to evaluate the management of the regional lymph nodes. A national intergroup protocol.
	NCIC ME.7	A clinical trial to compare immunomodulating doses of human interferon gamma with levamisole for the adjuvant treatment of poor prognosis malignant melanoma.
Colorectal Carcinoma	NSABP C-03	A clinical trial to compare adjuvant leucovorin and 5-FLU (LV-5-FU) with adjuvant MeCCNU, Vincristine and 5-FU (MOF) in patients with Duke's B and C carcinoma of the colon.
	NSABP C-04	A clinical trial to assess the relative efficacy of 5-FU and Leucovorin and Levamisole in patients with Duke's B and C carcinoma of the colon.
	NSABP R-02	A clinical trial to compare adjuvant MethylCCNU, Vincristine, 5-FU (MOF) with and without radiation in patients with Duke's B and C carcinoma of the rectum. (Females are excluded from this study).
	NCIC CO.3	To determine whether adjuvant treatment with 5-FU and folinic acid versus control is effective in increasing survivorship and prolonging the disease free interval in patients undergoing curative resection of colonic adenocarcinomas.
	NCIC CO.4	A clinical trial to determine the effect on survival of adjuvant therapy with 5-FU and Folinic Acid in patients who have had a curative resection of a carcinoma of the rectum and receive post-operative pelvic irradiation. Patients will be those who have a high risk of surgical treatment failure.
	MCR 85.9	To determine the efficacy and toxicity of Methotrexate and 5-FU (with Leucovorin rescue) in patients with advanced colon and rectal carcinoma.
	MCR 88.1	A clinical trial to study chemotherapy naive patients with metastatic rectal carcinoma to determine whether higher doses of epirubicin increase the efficacy of this drug in this tumor type.
Breast Carcinoma	NCIC MA.4	To compare Tamoxifen VS Tamoxifen plus combination chemotherapy in patients with ER or PR positive stage II breast carcinoma.

	NSABP B-16	A clinical trial comparing Tamoxifen alone VS L-PAM, 5-FU & Adriamycin & Tamoxifen or VS Short Intensive Adriamycin-Cyclophosphamide & Tamoxifen in positive node patients.
	NSABP B-17	Randomized study to evaluate post-operative breast irradiation following segmental mastec tomy plus axillary dissection in patients with non-invasive intraductal adenocarcinoma.
	NSABP B-19	A clinical trial to compare sequential Methotrexate 5-Fluorouracil with conventional Cyclophosphamide, Methotrexate and 5-FU in primary breast cancer patients with negative nodes and estrogen receptor negative tumors.
	NSABP B-20	A clinical trial to determine the worth of chemotherapy and Tamoxifen over Tamoxifen alone in the management of patients with invasive breast cancer, negative axillary nodes and estrogen receptor positive tumors.
	NSABP B-21	A clinical trial to determine the worth of Tamoxifen and the worth of breast radiation in the management of patients with node-negative, occult, invasive breast cancer treated by lumpectomy.
	NCIC IND.45	Phase II clinical trial of patients with evaluable metastic breast cancer utilizing oral menogaril; to determine efficacy and toxicity.
	MB 88.3	Epirubicin high dose VS conventional dose in metastic breast cancer. A randomized study in women after CMF failure.
Prostrate Carcinoma	PR 84.1	Ph. III randomized Megestrol Acetate plus low dose stilbestrol VS Diethylstilbestrol in Stage D^2 prostate cancer.
	PR 88.4	A comparison of Flutamide capsules VS Flutamide tablets in previously untreated prostate cancer patients.
	RTOG 85-31	Phase III study of Zoladex adjuvant to Radio-therapy in unfavourable prognosis carcinoma of the prostate.
	RTOG 86-10	Phase III study of Zoladex and Flatamide used as cytoreductive agents in locally advanced carcinoma of the prostate treated with definitive radiotherapy.

Pancreas Carcinoma	MPA 89.1	A clinical trial to assess survival and toxicity in patients with Stage 2, 3, or 4 of pancreatic carcinoma utilizing 5-FU and leucovrin.
Renal Carcinoma	NCIC IND.38	Phase II study of recombinant Tumor Necrosis Factor in metastatic renal cell carcinomas.
	NCIC IND.46	A phase II clinical trial in patients with metastatic renal cell carcinoma who have had no prior chemotherapy utilizing the drug LY186641 to determine efficacy and tumor response.
Metastatic Carcinoma	M.CA 85.8	To determine primarily toxicity, and secondly effectivenes of Lonidamine plus Interferon in metastatic carcinoma patients for whom there is no effective conventional treatment.
Symptom Control	NCIC SC.4	Double-blind assessment of the antiemetic efficacy, tolerance and safety of BRL &43694A in comparison with high dose metoclopramide dexamethasone and diphenhydramine in patients receiving highly emetogenic cisplatin chemotherapy.
	NCIC SC.5	Double-blind assessment of the antiemetic efficacy, tolerance and safety of BRL 43694A in comparison with dexamethasone and prochlorperazine in patients receiving moderately emetogenic chemotherapy.
	NCIC SC.6	A clinical trial to assess the antiemetic efficacy, tolerance, and safety of GR38032F versus Dexamethasone plus metoclopramide in patients with breast cancer receiving; CMF (cyclophosphamide, methotrexate, and 5-FU).
	NCIC SC.7	A double-blind clinical trial to assess the efficacy and safety of six doses of the antiemetic BMY-25801 and Methylprednisolone among patients receiving moderately emetogenic chemotherapy.
Staff Related Studies	NSABP	Health and Occupational Exposure to Anticancer Drugs.

PUBLICATIONS

Bow E.J., Louie, T.J. Emerging Role of Quinolones in the Prevention of Gram-Negative Bacteremia in Neutropenic Cancer Patients and in the Treatment of Enteric Infections. Clin. Invest. Med. Vol. 12, No. 1, pp. 61-68, 1989.

Dalal, B.I., Freier, L., Johnston, J.B., Merry, C.C. and **Israels, L.G.** Peripheral Blood and Bone Marrow Changes Following 2'Deoxycoformycin Therapy in Hairy Cell Leukemia. Cancer 63, 14-22, 1989.

Deffie, A.M., Batra, J.K. and **Goldenberg, G.J.** Direct Correlation Between DNA Topoisomerase II Activity and Cytotoxicity in Adriamycin-Sensitive and -Resistant P388 Leukemia Cell Lines. Cancer Research **49:**58-62, 1989.

Deffie, A.M., Bosman, D.J. and **Goldenberg, G.J.** Evidence for a mutant allele of the gene for DNA topoisomerase II in Adriamycin-Resistant P388 Murine Leukemia Cells. Cancer Res., **49:**6879-6882, 1989.

Deffie, A.M. and **Goldenberg, G.J.** Altered Levels of Expression of DNA Topoisomerase II in ADR-Senitive and -Resistant P388 Leukemia Cells. Proc. Am. Assoc. Ca. Res., 30, 514, 1989.

Fisher, B., Costantino, J., Redmond, C., Poisson, R., Bowman, D., Couture, J., Dimitrov, N.V., Wolmark, N., Wickerham, D.L., Fisher, E.R., Margolese, R., Robidoux, A., Shibata, H., erz, J., Paterson, A.H.G., Feldman, M.I., Farrar, W., Evans, J., Lickley, H.L., Ketner, M. A Randomized Clinical Trial Evaluating Tamoxifen in the Treatment of Patients with Node-Negative Breast ancer who have Estrogen-Receptor-Positive Tumors. N. Engl. J. Med., **320(8):**479-484, 1989.

Fisher, B., Redmond, C., Dimitrov, N.V., Bowman, D., Legault-Poisson, S., Wickerham, D.L., Wolmark, N., Fisher, E. R., Margolese, R., Sutherland, C., Glass, A., Foster, R., Caplan, R. A Randomized Clinical Trial Evaluating Sequential Methotrexate and Fluorouracil in the Treatment of Patients with Node-Negative Breast Cancer who have Estrogen-Receptor-Negative Tumors. N. Engl. J. Med., **320(8):**473-478, 1989.

Fisher, B., Redmond, C.R., Wickerham, L., Bowman, D., Schipper, H., Wolmark, N., Sass, R., Fisher, E.R., Jochimsen, P., Legault-Poisson, S., Dimitrov, N., Walter I., Bornsteen, R., lias, E.G., LiCalzi, N., Patterson, A.H.G., Sutherland, C.M. Doxorubicin-Containing Regimens for the Treatment of Stage II Breast Cancer: The National Surgical Adjuvant Breast and Bowel Project Experience. J. Clin. Oncol. 7:572-582, 1989.

Fisher, B., Redmond, C., Wickerham, D.L., Wolmark, N., Bowman, D., Couture, J., Demitrov, N.V., Margolese, R., Legault-Poisson, S., and **Robidoux, A.** Systemic Therapy in Patients with Negative-Node Breast Cancer: A Commentary Based on Two National Surgical Adjuvant Breast and Bowel Project (NSABP) Clinical Trials. Annals of Onternal Medicine. 111:703-712, 1989.

Jesson, M.I., Johnston, J.B., Robotham, E., and **Begleiter, A.** Characterization of the DNA-DNA Crosslinking Activity of 3'-(3-cyano-4-morpholinyl)-3'-deaminoadriamycin. Cancer Res., **49:**7031-7040, 1989.

Louie, T.J., Bow, E.J., Krulicki, W., Gray, G. Efficacy of Quinolones for the Prevention of Bacteremia in Neutroopenic Rats. Rev. Infect. Dis. **11(5):**S1242-S1252, 1989.

McNicol, A., Saxena, S.P., Brandes, L.J., and **Gerrard, J.M.** A Role for Intracellular Histamine in Ultrastructural changes Induced in Platelets by Phorbol Esters. Arteriosclerosis **9:**684-689, 1989.

Nickerson, P., Orr, P., Schroeder, M.L., Sekla, L. and **Johnston, J.B.** Tranfusion-Related Transmission of Trypansomiasis Cruzi in North America: A Case Report. Annals Int. Med. **111:**851-853, 1989.

Saxena, S.P., McNicol, A., Brandes, L.J., and **Gerrard, J.M.** A Role for

Intracellular Histamine in Collagen-Induced Platelet Aggregation. Blood **75**:407-414, 1989.

Schipper, H. Book Review: Quality of Life of Cancer Patients, J. Beckman and N.K. Aarnson, Journal of Palliative Care. Vol. **5**:3 1989.

Schipper, H. "How Palliative Care Improves Quality of Life" Expert Committee on Cancer Pain Relief Working Paper. World Health Organization, Geneva, July 3, 1989.

Schipper, H. The Montebello Conference; Goals and Recommendations." World Health Organization Bulletin, 1989.

Schipper, H. "The Montebello Conference". Journal of Palliative Care **5**:2 pp. 24-25, 1989.

Seneviratne, C. and Goldenberg, G.J. Further characterization of Drug-Sensitivity and Cross-Resistance Profiles of Cloned Cell Lines of Adriamycin-Sensitive and -Resistant P388 Leukemia.Cancer Communications, **1**:21-27, 1989.

Weinerman, B.H. and Orr, K.B. Colorectal Cancer—A Total Provincial Experience with Survival Analysis. Gan. J. Gastroenterol, Vol. 3, No. 3, June 1989.

Zanke, B.W., Rush, D.N., Jeffrey, J.R., Israels, L.G. HTLV-1 T cell Lymphoma in a Cyclosporine-Treated Renal Transplant Patient. Transplant 48, 695-697, 1989.

ABSTRACTS

Begleiter, A., Jesson, M.I., and **Johnston, J.B.** Crosslinking Activity of 3'-(3-cyano-4-morpholinyl)-3'deaminoadriamycin (CMA). Proc. Am. Assoc. Cancer Res., **30**:491, 1989.

Johnston, J.B., Israels, L.G., Goldebnerg, G.J., Mowat, M.R.A., and **Begleiter, A.** Relationship Between Glutathione S-Transferase (GST) Activity, Glutathione (GSH) Levels, and DNA Crosslinking (XL) Activity with Chlorambucil (CHL) in Chronic Lymphocytic Leukemia (CLL). Proc. Am. Assoc. Cancer Res., **30**:514, 1989.

Shepherd, J.D., Reece, D.E., Barnett, M.J., Bow, E.J., Klingemann, H.G., Shore, T.B., Phillips, G.L. Induction and Consolidation Therapy of Adult Acute Myelogenous Leukemia with High Dose Cytosine Arabinoside (HDARA-C) Etoposide (VP-16) and Daunorubicin (DNR). Leukemia/BMT Program of BC, Vancouver, BC & University of Manitoba, Winnipeg, Manitoba, Canada. IMEDEX, Bruistensingel 144, 5232 AC's-Hertogenbosch, The Netherlands, November 1989.

Weinerman, B,m MAcDougall, E., Carr, I. Departments of Medicine and Pathology St. Boniface Hospital and Manitoba Cancer Treatment and Research Foundation. "An Analysis of Patients Presenting with G. I. Non-Hodgkin's Lymphoma (NHL)". Presented Royal College of Physicians and Surgeons Meetings, Edmonton, Alberta September 22-25, 1989. Clin. Invest. Med. Vol. 12, No. 4, August 1989.

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Much of the basic research by the members of the Section of Paediatric Haematology/Oncology is carried out at the Manitoba Institute of Cell Biology. Research reports of this activity are presented under the Manitoba Institute of Cell Biology report. Studies with significant clinical relevance are covered here. These studies are presented as I) Abstracts of published papers and II) Active Clinical Studies.

I. Abstracts of published papers

A REASSESSMENT OF THE BLEEDING TIME: ASSOCIATION OF AGE, HEMATOCRIT, PLATELET FUNCTION, VON WILLEBRAND FACTOR, AND BLEEDING TIME THROMBOXANE B_2 WITH THE LENGTH OF THE BLEEDING TIME

J.M. Gerrard, J.C. Docherty, S.J. Israels, M.S. Cheang, A.J. Bishop, N.L. Kobrinsky, M.L. Schroeder, and E.D. Israels

In order to provide an overview of the relative contribution of platelet, von Willebrand factor, and other abnormalities to patients with clinical bleeding difficulties, we performed a retrospective survey of coagulation studies on 569 individuals referred to the University of Manitoba coagulation laboratory because they, or a closely related family member, showed clinical evidence of a bleeding disorder. There was a highly significant ($p<0.001$) negative correlation between the bleeding time and each of the following parameters: the platelet count; the hematocrit; the percent aggregation to collagen, epinephrine, ADP, and arachidonic acid; and the logarithm of von Willebrand factor antigen and a measure of its activity (ristocetin cofactor). A significant and independent inverse relationship between the length of the bleeding time and the extent of platelet adhesion to glass beads, patient age, and prothrombin consumption were also observed. Multivariate analysis of the ability of all parameters to predict the bleeding time showed an r^2 of only 0.33. Bleeding time thromboxane B_2, in a second smaller study of 70 patients, showed a negative correlation with the length of the bleeding time (p - 0.0001). When thromboxane B_2 was used together with the above parameters, this significantly enhanced the ability to predict the length of the bleeding time (r^2 - 0.55). Defects in platelet function, as measured in vitro, and significant enough to have an effect on the bleeding time, occurred with greater frequency than defects in von Willebrand factor in the Manitoba patients evaluated.

IN VIVO MEASUREMENT OF THROMBOXANE B_2 AND 6-KETO-PROSTAGLANDIN $F_{1\alpha}$ IN HUMANS IN RESPONSE TO A STANDARDIZED VASCULAR INJURY AND THE INFLUENCE OF ASPIRIN

J.M. Gerrard, S. Taback, S. Singhroy, J.C. Docherty, I. Kostolansky, A. McNicol, N.L. Kobrinsky, J.K. McKenzie and R. Rowe

The effects of smoking, aspirin ingestion, and sex differences on bleeding times and bleeding time thromboxane B_2 and 6-keto-prostaglandin (PG)$F_{1\alpha}$ production were examined. Nonsmoking men produced more thromboxane B_2 (3.99±0.76 ng/ml) than nonsmoking women (2.13±0.24 ng/ml). Female smokers produced more thromboxane B_2 (5.01±0.97 ng/ml) than nonsmoking women. Twenty-four hours after a single dose of 600 mg aspirin, in vitro production of thromboxane B_2 in response to collagen fell by 95%, whereas in vivo production of thromboxane B_2 and 6-keto-$PGF_{1\alpha}$ in bleeding time blood fell by 87%

and 66%, respectively. Subjects with the lowest absolute levels of thromboxane B_2 24 hours after aspirin were also those with the longest postaspirin bleeding times. Recovery of 6-keto-$PGF_{1\alpha}$ production for most subjects was still below basal 72 hours after aspirin. The influence of two different doses of long-term aspirin (80 mg every other day and 325 mg daily) on the in vivo production of thromboxane B_2 and 6-keto-$PGF_{1\alpha}$ as studied in normals and diabetics. After 14 days of 80 mg aspirin every other day, thromboxane B_2 and 6-keto-$PGF_{1\alpha}$ production were both substantially inhibited (93% and 78%, respectively). After 14 days of 325 mg aspirin daily, thromboxane B_2 production was similarly substantially inhibited (93%), whereas 6-keto-$PGF_{1\alpha}$ was significantly less affected (only 45% inhibition). Study of a second group of five normal subjects confirmed that 6-keto-$PGF_{1\alpha}$ production was significantly inhibited 24 hours after the first dose of 325 mg aspirin but was not significantly less than basal after 14 days of 325 mg aspirin. The results suggest that 325 mg aspirin daily is more antithrombotic compared with 80 mg every other day due to the superior preservation of prostacyclin production.

MEASUREMENTS OF 6-KETO-PROSTAGLANDIN $F_{1\alpha}$ AND THROMBOXANE B_2 IN BLEEDING TIME BLOOD: RELATION TO BLEEDING AND VASCULAR DISORDERS?

J.M. Gerrard

The body's ability to produce prostacyclin and thromboxane by blood vessels and platelets may be important in hemostatic and thrombotic disorders and in blood pressure regulation. There are limitations to the information that can be derived from measurement of the active substances or metabolites in plasma and urine. Assays for thromboxane and prostacyclin in bleeding time blood reflect production in response to a single standardized vascular injury, and show considerable promise in furthering our understanding of the production of these chemicals in vivo. These assays may improve the assessment of risk of developing thrombotic disorders and improve the ability to monitor treatment. Studies to date have focused largely on the influences of various doses of aspirin on the production of prostacyclin and thromboxane in bleeding time blood, but also suggest that smokers are high thromboxane producers. In addition, individuals who exhibit type A behavior, a behavior pattern characterized by a relatively high level of ambitiousness, hostility, and competitive drive and a chronic sense of urgency appear to be low prostacyclin producers. Diets enriched in sunflower oil were found to diminish thromboxane production, while diets high in canola oil enhanced prostacyclin formation.

THE EFFECT OF EXERCISE ON BLEEDING TIME AND LOCAL PRODUCTION OF PROSTACYCLIN AND THROMBOXANE

J.W. Carter, A.E. Ready, S. Singhroy, E. Duta and J.M. Gerrard

Exercise at a heart rate corresponding to 30% Vo_{2max} for 15 min was associated with an increase in the volume of bleeding time blood from a mean of 133 ul before exercise to a mean of 218 ul during and immediately after the exercise. There was similarly an increase in thromboxane B_2 production from 6.40 $nmol.1^{-1}$ before to 11.50 $nmol.1^{-1}$. Most subjects also showed an increase in the length of the bleeding time and in the production of bleeding time 6-keto-$PGF_{1\alpha}$. The extent of increase in the bleeding time and in production of 6-keto-$PGF_{1\alpha}$ was quite variable, with subjects showing the largest increases in bleeding time

also demonstrating the greatest increases in 6-keto-$PGF_{1\alpha}$(r-0.76, P-0.004). The ingestion of aspirin before exercise markedly inhibited basal bleeding time thromboxane B_2 production and blocked the exercise-associated increments in thromboxane B_2 and 6-keto-$PGF_{1\alpha}$ production. While the aspirin itself increased the length of the bleeding time, there was not any further increase associated with exercise. In contrast to the effects of acute short-term exercise, long-distance running was associated with a significant decrease in bleeding time, but no change in bleeding time blood volume, bleeding time thromboxane B_2, or bleeding time 6-keto-$PGF_{1\alpha}$ The results show that acute low-level exercise can be associated with significant changes in the volume of blood oozing from a bleeding time incision and in the amount of thromboxane production stimulated at the incisional site. Following exhaustive exercise of long duration, the above changes are no longer seen.

HEMATOLOGY EXPERT SYSTEM (HES) FOR TONSILLECTOMY/ ADENOIDECTOMY PATIENTS

N.J. Pizzi, S. Kapoor and J.M. Gerrard

The purpose of this expert system is to assess a predisposition to bleeding in a patient undergoing a tonsillectomy and/ or adenoidectomy as may occur with patients who have certain blood conditions such as hemophilia and von Willebrand's disease. This goal is achieved by establishing a correlation between the patients' responses to a medical questionnaire and the relative quantities of blood lost during the operation.

There are three major components to the system. First, there is an automated questionnaire to be completed by the patient (or the patient's guardian) which covers bleeding/bruising symptoms and the family history of such symptoms. Secondly, there is the expert system proper which takes the responses to the questionnaire and determines risk categories for patients undergoing the operations. The expert system also uses any coagulation lab data that may have been recorded for the patient (this data is sometimes present when a patient definitely has [or is suspected to have] some blood disorder and is subsequently referred to the hematology section). Finally, there is a patient database which contains the patients' questionnaire responses, personal data, medical data (if any were recorded), and the final and intermediate risk assessments generated by the expert system. Standard statistical analysis techniques are applied to the patient records in the database to determine the sensitivity and specificity of the expert system's risk assessments as compared against the patient's blood loss during the operation and, if warranted, an off-line update of the system's knowledge base may then be performed.

This paper discusses the knowledge architecture of the hematology system, identifies some problems encountered during the knowledge engineering process and presents some statistical data pertaining to the accuracy of its risk assessments.

COMPARISON OF THE EFFECT OF CANOLA OIL AND SUNFLOWER OIL ON PLASMA LIPIDS AND LIPOPROTEINS AND ON IN VIVO THROMBOXANE A_2 AND PROSTACYCLIN PRODUCTION IN HEALTHY YOUNG MEN

B.E. McDonald, J.M. Gerrard, V.M. Bruce and E.J. Corner

The effect of dietary fatty acid composition on plasma lipids and eicosanoid production was studied in normolipidemic men. Approximately 75% of the fat in the diet was provided by a mixture of fats (MF) during the 6-d pre

and postperiods and either canola oil (CO) or sunflower oil (SO) during the two 18-d experimental periods. Saturated, monounsaturated, and polyunsaturated fatty acids provided 14%, 15%, and 7%; 20%, and 10%; and 7%, 7%, and 22%, respectively, of total dietary energy in the MF, CO, and SO diets, respectively. The CO and SO diets produced similar decreases in plasma total (20% and 15%, respectively) and low-density lipoprotein (25% and 21%, respectively) cholesterol. Plasma high-density lipoprotein cholesterol and triglyceride concentrations were not altered by either fat source. Bleeding time was longer and in vivo 6-keto-$PGF_{1\alpha}$ production was greater ($p<0.05$) when on the CO diet than when on the MF diet. The hypocholesterolemic and antithrombotic effects of the CO diet were equivalent to those of the SO diet.

FATTY ACID COMPOSITION OF UMBILICAL ARTERIES AND VEINS: POSSIBLE IMPLICATIONS FOR THE FETAL EFA-STATUS

G. Hornstra, A.C. van Houwelingen, M. Simonis and J.M. Gerrard

Fatty acid compositions were determined of phospholipids, isolated from umbilical arteries and veins, obtained from Dutch neonates after vaginal delivery, terminating normal pregnancy. The fatty acid profiles of the cord vessels were characterized by the absence of eicosapentaenoic (timnodonic) acid, a low (2-3%) content of linoleic acid and reasonable amounts of arachidonic acid (10-15%) and docosahexaenoic (cervonic) acid (3-5%). Significant amounts of Mead acid (1-4%) and its direct elongation product (0.5-2%) were also observed. In each cord, the efferent blood vessels contained significantly more Mead acid and other fatty acids of the oleic acid (n-9) family and less fatty acids of the linoleic (n-6) and linolenic (n-3) families than the afferent blood vessel. This indicates that the essential fatty acid (EFA) status of 'downstream' neonatal tissue may be marginal. No signs of EFA-deficiency were observed in endothelial and smooth muscle cells in culture, or in blood vessels from adults. In all cords (22:5(n-6) was significantly higher in the artery compared to the vein, whereas for all other (n-6) fatty acids this difference was negative. Since the synthesis of 22:5(n-6) is known to be stimulated when the required amount of cervonic acid, 22:6(n-3), is too low, our observations also suggest that the cervonic acid status of the neonates investigated was not optimal. Further studies are in progress to relate these findings to maternal EFA status and complications of pregnancy.

II. Active Clinical Studies

Canadian Multicentre Study of Childhood Leukemia and Low Frequency Electromagnetic Field Exposure

Leukemia is the most common childhood malignancy in Canada. The etiology of leukemia is not well understood and several recent studies have suggested that children exposed to strong low frequency electrical or magnetic fields may be at higher risk of this cancer.

Previous studies, however, have suffered from two substantial limitations: inability to accurately quantify electromagnetic field exposure, and lack of sufficient numbers of adequately classified cases to adequately determine any relationship among different subtypes of leukemia.

The risk of all childhood leukemia and each subtype of leukemia in the presence of 60 hertz electromagnetic

field exposure will be examined using the following criteria:

a) indirect measurement of electromagnetic field (EMF) exposure in current and all previous residences, using wire configuration data.

b) exposure to 60 hertz magnetic and electrical field as measured by 24 hour dosimetry of the child's bedroom in the first year of life. This measurement will be taken on a subset of the total study cohort, depending on availability. Average exposure, duration of high exposure, and various components of electromagnetic field exposure can be analyzed separately from this data.

c) exposure to 60 hertz magnetic and electrical field as measured by personal dosimetry on a 48 hour basis. This measurement will be restricted to subjects still alive at time of data collection.

As well, we will assess the possible association of the following groups of factors with total childhood leukemias, and each leukemia subtype: family history of cancer and congenital anomalies; parental occupational and recreational exposures, from the year prior to the birth of the subject to the time of diagnosis; gestational factors and other exposures surrounding pregnancy; post-natal factors, including radiation, and exposure to viral infections, chemicals, and medications; and socioeconomic status.

A total of 400 parents of children with leukemia will be interviewed with a standardized questionnaire, along with an equal number of control parents with children of the same sex matched for year of birth. All interviews will be conducted when the case children's disease is in remission.

Canadian Multicentre ITP Trials

This multicentre Canadian study is evaluating the response of children with acute ITP with platelet counts less than 20 x 10^9/L just for therapeutic regimes. These include prednisone 4 mg/k/day, intravenous immunoglobulin 1 gr/k/day x 2 days, intravenous immunoglobulin 0.8 gr/day x 1, anti-D 25 µg/k/day x 2 days. This study is to evaluate the rate of response to platelet count and any side effects associated with the various therapies. This study opened in the Fall of 1988 and there has been steady accrual of patients since that time. The goal for statistical purposes is to enroll 150 patients over three years. This study's objective is to identify optimal therapy for acute ITP, both in terms of rate of response and incidence of side effects.

CCG-7881 — EVALUATION OF VINCRISTINE, ADRIAMYCIN, CYCLOPHOSPHAMIDE AND ACTINOMYCIN, WITH OR WITHOUT THE ADDITION OF IFOSFAMIDE AND ETOPOSIDE IN THE TREATMENT OF PATIENTS WITH NEWLY DIAGNOSED EWING'S SARCOMA OR PRIMITIVE NEUROECTODERMAL TUMOR OF BONE. A PHASE III INTERGROUP STUDY

In this study a control regimen of high dose intermittent pulses of vincristine, adriamycin and cyclophosphamide (VAC) is compared with an experimental regimen using courses of ifosfamide and etoposide, alternating with VAC. In both regimens adriamycin is replaced with actinomycin D, when the total cumulative dose of adriamycin reaches 375 mg/M^2 in order to prevent anthracycline-induced cardiotoxicity. In both regimens, local therapy includes intensive radiation therapy to a total dose of 5580 cGy using 31 fractions of 180 cGy/fraction.

CCG-631 — THE INTERGROUP RHABDOMYOSARCOMA STUDY III

The IRS III study is based on the results obtained from the IRS I and II studies. Patients with favourable histology group I tumors will receive cyclic sequential vincristine and actinomycin-D with no radiation. Patients with groups I or II unfavourable histology will receive vincristine, adriamycin, cis-platinum, cyclophosphamide induction and pulse-VACdC, alternating with pulse-VAC maintenance. Radiation to the tumor bed will also be administered to unfavourable histology patients. Patients with group II favourable histology will be randomized to receive cyclic-sequential vincristine and actinomycin-D with or without adriamycin; all patients to receive radiation to the tumor bed. Patients with group II or III tumors involving the orbit or non-parameningeal sites of the head will not be randomized, but will receive local radiation and cyclic-sequential vincristine, actinomycin-D only. Patients with group III and group IV tumors will be randomized to receive an induction chemotherapy with pulse-VAC or two experimental arms containing vincristine, actinomycin-D, cyclophosphamide, cis-platinum, adriamycin ± VP-16. Additional strategies for group III and IV patients will include second and third look surgeries and non-cross-resistant intensification therapies with adriamycin/DTIC, high dose actinomycin-D/VP-16, or high dose actinomycin-D/DTIC. Patients with primary parameningeal sites will have their cranial prophylaxis stratified according to the potential risk of CNS extension. Patients with genitourinary primaries will receive intensive primary chemotherapy and delayed radiation and surgery in an attempt to decrease therapy-related morbidity.

CCG-671 — RHABDOMYOSARCOMA STUDY IV, PILOT STUDY FOR CLINICAL GROUP III DISEASE

The IRS-IV pilot proposes to treat patients with ifosfamide, actinomycin-D and vincristine, followed by hyperfractionated radiation therapy, followed by a maintenance therapy with ifosfamide, actinomycin-D and vincristine. Assuming that the results of the pilot study are acceptable, the IRS-IV, stage III randomized study will proceed. Patients will be randomized between three chemotherapy arms, including vincristine, actinomycin-D and cyclophosphamide; vincristine, actinomycin-D, ifosfamide; and vincristine, etoposide and ifosfamide. Patients will also be randomized to receive standard radiation therapy to a total dose of approximately 5,000 cGy, or hyperfractionated radiation therapy to a total dose of approximately 6,000 cGy.

CCG-6881 — INTERGROUP RHABDOMYOSARCOMA STUDY IV PILOT STUDY FOR CLINICAL GROUP IV DISEASE

As accrual on IRS-III has exceeded expectations, patients with Stage IV disease will be closed to further patient entry on this protocol. The IRS committee has therefore recommended enrollment of stage IV patients henceforth on a pilot study which will be used in the development of the forthcoming open, randomized IRS-IV trial. The IPS-IV pilot study for stage IV disease is based on the known poor outcome for this group of patients (long term disease-free survival of 20-27%), as well as a number of new developments in the treatment of soft tissue sarcomas. These developments include the "discovery" of ifosfamide and of melphalan as effective agents in the treatment of soft tissue sarcomas and the recognition that

hyperfractionated radiation therapy may provide better local disease control than standard radiation therapy. The IRS-IV, stage IV pilot will treat patients with the combination of vincristine, doxorubicin and ifosfamide. After an initial induction phase, patients will receive hyperfractionated radiation therapy, followed by a maintenance with alternating courses of vincristine, ifosfamide, doxorubicin and subsequently vincristine, actinomycin-D, cyclophosphamide (VAC). For patients who do not show an initial favourable response to the vincristine, ifosfamide, doxorubicin combination, cross-over to vincristine, actinomycin-D, cyclophosphamide will take place.

The IRS-IV, stage IV pilot study, if acceptable with regard to disease response and toxicity will then be expanded into a group-wide randomized trial. The trial will compare vincristine, ifosfamide, doxorubicin and vincristine, melphalan with vincristine, ifosfamide, etoposide. This study will also be randomized to standard versus hyperfractionated radiation therapy. The vincristine, ifosfamide, doxorubicin combination has been chosen for pilot evaluation, since the vincristine, melphalan combination has been already piloted by the St. Jude's group, and because the vincristine, ifosfamide, etoposide combination is to be piloted concurrently for patients with stage III disease (CCG-671). In both the pilot and the open study patients who do not respond to initial therapy will be assigned to treatment with standard vincristine, actinomycin-D and cyclophosphamide (VAC). This will provide information concerning cross-resistance between the three experimental induction therapies and VAC standard induction therapy.

Protocol NWTS-4

The NWTS-4 hopes to further refine the treatment for patients with Wilms tumor. The two main questions include whether Actinomycin-D, when administered in a 'pulse-intensive' fashion will be superior to the five day approach. Clearly, the pulse-intensive therapy will be more convenient for the patients, but more importantly, should have some theoretical benefit because of its increased intensity. The length of therapy required for the various stages and histological subtypes will also be further refined. It is hoped that the NWTS-4 will define the "state of the art" treatment for patients with Wilms tumor, and in this respect, is a very important study. The question of ideal therapy for patients with the "rhabdoid tumor" and patients with stage II, III and IV anaplastic tumors, of course, will not be defined by this study, but hopefully some information regarding optimal treatment for these patients will still emerge from on-going accrual on NWTS-3.

CCG-8881— AN INTERGROUP PROTOCOL FOR THE TREATMENT OF CHILDHOOD HEPATOBLASTOMA AND HEPATOCELLULAR CARCINOMA. A PHASE III GROUP WIDE STUDY.

All patients with completely resected disease who have been confirmed to have a favorable histology will receive adriamycin given as i.v. bolus at the dose of 20 mg/M²/day for three consecutive days. Cycles will be repeated at 3 to 4 week intervals for a total of four cycles.

Children with hepatoblastoma will be divided into two groups: a favorable histology Stage I group to receive doxorubicin and all other children with the disease who will be randomized to receive either Cisplatin-Adriamycin i.v.

continuous infusion or Cisplatin-Vincristine-5-Flurouracil treatment. Evaluation of alphafetoprotein and serum ferritin will provide evidence of the utility of these markers in this disease.

CCG-8882 — A STUDY OF HIGH-RISK MALIGNANT GERM CELL TUMORS IN CHILDREN

Following collection of appropriate pathological and biochemical data, patients will be randomized to receive either high-dose cisplatin, etoposide, and bleomycin (Arm 1), or 2) regular dose cisplatin, etoposide, and bleomycin induction (Arm 2) to begin as soon as possible but within no later than 21 days of the most recent diagnostic surgical procedure. In each case induction will be given in weeks 0, 3, 6, and 9 with maintenance therapy to be given in weeks 13 and 16 for patients who remain in partial remission following post-induction surgery or who are converted to complete remission with post-induction surgery. Patients found to be in complete remission as a result of chemotherapy alone with no viable disease at post-induction surgery (week 12) will not receive maintenance chemotherapy. Those with no response will be off the protocol therapy.

CCG-321P1 — INTENSIVE MULTIMODAL THERAPY AND BONE MARROW TRANSPLANTATION FOR NEWLY DIAGNOSED STAGE IV, AND POOR RISK STAGE III NEUROBLASTOMA PATIENTS. PHASE III STUDY

For all tumors studied to date, bone marrow transplantation has been most successful when performed at a time of minimal tumor burden prior to disease progression. For this reason, treatment with standard chemotherapy will be administered at three week intervals for four courses from the time of initial diagnosis (weeks 0, 3, 6 and 9). This will be followed by a second look surgical procedure to remove any gross residual disease, followed immediately by local irradiation to residual unresectable tumor sites. This cytoreductive regime will then be followed shortly thereafter by intensive non-cross-resistant chemotherapy and bone marrow transplantation. The induction chemotherapy that will be used will be the standard therapy that has been used for neuroblastoma for the past ten years with cyclophosphamide, vincristine and DTIC. With this therapy, maximal responses are usually obtained by weeks 10-13, i.e., after the fourth or fifth courses of chemotherapy. Further benefit has not been demonstrated by continuing this therapy for a longer period of time; in fact, tumor progression has been observed in 15, 25, 40 and 50% of patients by 9, 12, 15 and 22 weeks, respectively. Therefore, after the first four courses of chemotherapy, second-look surgery and local irradiation will be performed at week 11, with transplantation at week 13.

The CCG-321P1 protocol is only available for patients who have an HLA-compatible sibling donor. Patients who do not have a donor will, therefore, be treated on an analogous protocol using repeated cycles of less intensive chemotherapy without transplantation (CCG-321P2).

CCG-3881 — BIOLOGY AND THERAPY OF GOOD, INTERMEDIATE, AND SELECTED POOR PROGNOSIS NEUROBLASTOMA

Four groups of patients with neuroblastoma are to be defined and treated according to the following protocols.

Patients with stages I and II disease and less than three copies N-myc will be treated with surgery alone, unless there is symptomatic cord compression, in

which case, children with rapidly progressive neurologic defects are to be treated with surgery with the possible addition of radiation therapy in lesions of a more chronic unstable nature.

Patients with stage II, III and IV and unfavorable prognostic signs will be treated on other protocols. This will include patients who are stage II greater than one year of age and have greater than or equal to three copies of N-myc, patients with stage III greater than one year of age and unfavorable histopathology, greater than or equal to three copies of N-myc or elevated ferritin, and patients with stage IV who are greater than one year of age.

For the patients with stages II with greater than or equal to three copies of N-myc and patients with stages III and IV who are to be treated under the current protocol, following diagnostic biopsy or subtotal tumor resection, they will receive a 120-day induction phase of chemotherapy consisting of cyclophosphamide, cisplatinum, adriamycin and VP-16 (constant infusion). At day 120, patients are evaluated for delayed surgery. An attempt will be made to resect previously unresectable tumor or to debulk residual disease. Radiation therapy is given to tumors that do not appear to be amenable to surgical debulking, as well as to tumor persisting after surgery. Following completion of radiation therapy, patients are to be given a 113-day course of chemotherapy, utilizing the same agents as those used in the induction phase. Patients with residual disease remaining after surgery at day 120, are surgically explored and metastatic foci biopsied to determine whether or not active disease is present. Patients having no evidence of disease are observed without further therapy, while those who have histologic evidence of persistent disease go on to alternate therapy.

CCSG 921— PROTOCOL FOR THE TREATMENT OF UNFAVOURABLE MEDULLOBLASTOMA AND INTERCRANIAL PRIMITIVE NEUROECTODERMAL TUMORS (PNET), MALIGNANT EPENDYMOMA, EPENDYMOMABLASTOMA, PINEOBLASTOMA, AND CENTRAL NEUROBLASTOMA, A PHASE III MULTI-INSTITUTIONAL STUDY

The proposed CCG-921 study is based on favourable results obtained in the previous CCG-942 study, as well as the very exciting preliminary data obtained from the "8 in 1" pilot program. The study calls for a randomization between the chemotherapy-containing arm of the CCG-942 using the three drugs, CCNU, vincristine and prednisone, and the "8 in 1" program. Radiation therapy is to be standardized in both treatment arms. In the control arm vincristine is administered weekly during the radiation therapy phase and maintenance chemotherapy follows the radiation therapy for approximately one year. In the "8 in 1" arm, two courses of "8 in 1" are administered prior to radiation therapy and eight courses are administered after radiation therapy.

CCSG-945 — PROTOCOL FOR THE TREATMENT OF HIGH GRADE INTERCRANIAL ASTROCYTOMA PREVIOUSLY UNTREATED, LOCATED OUTSIDE THE BRAIN STEM OR SPINAL CORD

The CCG-945 is a multi-institutional phase III trial whereby patients are randomized between surgery, conventional radiation therapy (5500 rads to the extended involved field, but not whole brain) and either the 3 drug regime used in the CCG-943, or the 8 drug regime used in the "8 in 1" pilot. The drugs in the "8 in 1" include methylprednisolone,

CCNU, procarbazine, hydroxyurea, cytosine arabinoside, DTIC, vincristine and cis-platinum. The drugs are administered over a 16 hour period of time.

CCG-9881 — EVALUATION OF VP-16-213 (ETOPOSIDE, NSC 41540) WITH OR WITHOUT MANNITOL FOR THE TREATMENT OF RECURRENT OR PRIMARILY UNRESPONSIVE BRAIN TUMORS, A RANDOMIZED CLINICO-PHARMACOLOGIC PHASE II GROUPWIDE STUDY

Patients enrolled in the study will be randomized to receive VP-16-213 at an optimal five day dosage schedule with or without the hyperosmolar sugar mannitol. If the results observed in the pilot study are due to the addition of the hyperosmolar sugar, then an advantage for the mannitol-containing arm should be demonstrated. Theoretical concerns with the mannitol-containing arm would include the possibility that mannitol may open the blood/brain barrier throughout the brain in both normal and abnormal tissue and therefore increase potential neurotoxicity. This has not been seen to date.

CCG-9882 — HYPERFRACTIONATED RADIATION THERAPY IN PEDIATRIC BRAIN STEM GLIOMAS. PHASE I, PHASE II GROUP-WIDE PILOT STUDY

The present study proposes to evaluate the use of hyperfractionated radiation therapy in a group-wide setting for pediatric patients with poor risk brain stem gliomas. These tumors do not require histologic confirmation, unless they are exophytic, in which case anaplasia must be demonstrated. Fifty patients will be treated at a dose of 100 cGy twice daily, to totals of 7,200 cGy as reported by the San Francisco group. The study will be closed prior to accruing 50 patients if undue toxicity is encountered. If 50 patients complete the therapy without excessive acute toxicity, then the second phase of the study will evaluate a higher radiation dose of 100 cGy twice daily to a total dose of 7,800 cGy. In the event of a fatal outcome, post mortem examinations are strongly requested, to ensure the deterioration was not due to brain stem necrosis.

CCG-9883 — INTENSIVE CHEMOTHERAPY AND AUTOLOGOUS BONE MARROW RECONSTITUTION FOR CHILDREN WITH RESISTANT MALIGNANT BRAIN TUMORS

Patients under 25 years of age with malignant brain tumors (e.g., anaplastic astrocytoma, glioblastoma multiforme, medulloblastoma, high-grade ependymoma, pineoblastoma, germ cell tumors and other primitive neuroectodermal tumors) that have recurred or have proven resistive to conventional therapies (surgery, radiation therapy, standard multi-agent chemotherapy regimens), and who have not previously been exposed to either thiotepa or VP-16 at high doses are eligible. Patients to be entered in the study must also have no evidence of marrow involvement with tumor on bilateral posterior iliac crest bone marrow biopsies and aspirates. The treatment plan will include thiotepa and VP-16 given 5 days, 4 days, and 3 days before autologous bone marrow infusion. Any patient who develops progressive disease following intensive chemotherapy and marrow reinfusion on the study will go off protocol therapy but will be followed for ultimate survival and for late toxicities. Any patient who has bulky unresectable disease before bone marrow transplant and who has residual disease still evident 2 months after bone

marrow transplant may receive involved field radiation therapy at the discretion of the investigator.

CCG-1891— TREATMENT OF NEWLY DIAGNOSED ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN WITH AN INTERMEDIATE PROGNOSIS*

Regimen A — This regimen, a standard therapy, is based on preliminary results from the previous Children's Cancer Study Group study (CCGS-105) in which patients will receive induction therapy including Vincristine, Prednisone, L-Asparaginase and intrathecal Methotrexate. Induction is then followed by consolidation with 6-Mercaptopurine, intrathecal Methotrexate, Vincristine, and Prednisone for four weeks. Consolidation is followed by interim maintenance I of four weeks' treatment with 6-Mercaptopurine, Methotrexate, Vincristine and Prednisone and then eight weeks of delayed intensification including Vincristine, Dexamethasone, Adriamycin, L-Asparaginase, Cyclophosphamide, Cytosine Arabinoside, 6-Thioguaine and intrathecal Methotrexate. Up to this point, all regimens are identical. It is at this point that differences in the three regimens begin. In regimen A, maintenance therapy will be Vincristine and Prednisone every 28-days combined with daily 6-Mercaptopurine and Methotrexate together with intrathecal Methotrexate once every 12-weeks. Maintenance therapy will continue for 88-weeks for girls and for 140 weeks for boys.

Regimen B — Regimen B is identical to regimen A to the end of the delayed intensification. At this point, children enrolled in regimen B will receive 8-weeks of interim maintenance with 6-Mercaptopurine, Methotrexate, Vincristine and Prednisone followed by an 8-week delayed intensification repeat using Vincristine, Dexamethazone, Adriamycin, L-Asparaginase, Cyclophosphamide, Cytosine Arabinoside, 6-Thioguaine and intrathecal Methotrexate. After the delayed intensification, children will receive maintenance with Vincristine and Prednisone every 28-days together with daily 7-Mercaptopurine and Methotrexate, and intrathecal Methotexate every 12-weeks. This maintenance will go for 72 weeks in girls and 124 weeks in boys before therapy is discontinued.

Regimen C — This third regimen, regimen C, is similar to regimen A in that Vincristine and Prednisone are to be used, but they are to be given every 21 days instead of every 28 days. 6-Mercaptopurine and Methotrexate are to be given daily and intrathecal Methotrexate is to be given once every 12-weeks as in regimen A.

Below, very briefly, the rationale for the variations chosen in the standard therapy are as follows:

Regimen B attempts to evaluate whether a second delayed intensification is superior to a single one. Regimen C attempts to determine whether Vincristine and Prednisone given on a 21-day schedule is superior to Vincristine and Prednisone given on a 28-day schedule.

*(In patients aged 1-2 years with white blood counts <10,000/ul, patients aged 1-10 years inclusive with white blood counts of 10,000 to 50,000/ul, and boys aged 1-10 with white blood counts of ≤10,000/ul and platelets of <100,000/ul, excluding patients with lymphoma-leukemia and/or L3 morphology).

CCG-1881 — TREATMENT OF NEWLY DIAGNOSED LOW RISK ACUTE LYMPHOBLASTIC LEUKEMIA. A PHASE III GROUP WIDE STUDY

To summarize, this study will attempt to determine whether the addition of a delayed intensification therapy will improve the outlook for children with low risk acute lymphocytic leukemia. Inten-

sive therapy would potentially be associated with approximately a 2% mortality; however, with between 15 and 20% of patients relapsing on current therapy, this seems to be a reasonable trade-off if the therapy is effective.

CCG-1882 — TREATMENT OF NEWLY DIAGNOSED ACUTE LYMPHOCYTIC LEUKEMIA IN CHILDREN WITH A POOR PROGNOSIS EXCLUDING INFANTS AND PATIENTS WITH LYMPHOMA OR FAB L3 BLASTS. A PHASE III GROUP WIDE STUDY

This study will attempt to:

a) Determine that patients showing a good response by day 7 of chemotherapy, with BFM without cranial RT will show decreased morbidity without an alteration in event free survival when compared with standard BFM.

b) Improve even free survival of those children treated with BFM showing a poor response on day 7 by treating them with intensified chemotherapy with minimal increase in toxicity.

c) Further study the interaction of transitional prognostic factors; day 7 marrow response, cytogenetics, and immunophenotype on outcome.

The Manitoba Cancer Foundation may enroll 2 patients per year.

CCG-1883 — TREATMENT OF NEWLY DIAGNOSED ACUTE LYMPHOBLASTIC LEUKEMIA IN INFANTS LESS THAN 12 MONTHS OF AGE. A PHASE III GROUP WIDE STUDY

To summarize, CCG-1883 will build on the encouraging results of CCG-107. It is a non-randomized study, which will have the following features:

1. Intensive four-drug induction.
2. Consolidation with high-dose Ara-C and L-asparaginase, as well as very high dose methotrexate with leucovorin rescue and intrathecal chemotherapy.
3. Interim maintenance regimen with vincristine, moderate dose methotrexate and L-asparaginase (Capizzi 1).
4. Delayed intensification therapy, which, in fact, is a truncated version of the induction and consolidation, and based on the theoretical principles delineated by the BFM protocol.
5. "Standard" maintenance therapy.

A very significant feature of this protocol will be the prospective performance of psychoneurologic testing to confirm the initial impression that by eliminating central nervous system radiation, long term CNS sequelae have been reduced, or perhaps even eliminated.

CCG-1884 — A COMPARISON OF IDARUBICIN TO DAUNOMYCIN IN A MULTI-DRUG TREATMENT OF ALL IN MARROW RELAPSE. A GROUP WIDE STUDY

The proposed study will attempt to improve disease control in this high risk group of patients by several therapeutic strategies. A four drug induction with vincristine, prednisone, L-asparaginase and an anthracycline will be used. Patients will be randomized to one of two anthracyclines, daunomycin or idarubicin. Daunomycin has been used for the past ten years as an effective antileukemic agent. Idarubicin is an analog of daunomycin developed by a deletion of the methoxy moiety on the aglycone ring. Idarubicin appears to be more effective than daunomycin as a single agent. It is less cardiotoxic and may be used orally. Patients who do not achieve remission with this four drug induction will receive a "salvage" therapy with high dose Ara-C and L-asparaginase rescue.

This regimen ("Capizzi II") has been effective in the treatment of both acute lymphoid and myeloid leukemias in children and in adults. In the pilot study to CCG 1884, 4 of 8 patients who failed to achieve a remission with the four drug induction did successfully go into remission with the Capizzi II regimen. Patients who enter remission after the four drug induction only will then receive two courses of moderate dose methotrexate with L-asparaginase rescue ("Capizzi I"). Patients with an HLA identical donor will then proceed to bone marrow transplantation using either the Cleveland (high-dose Ara-C with fractionated total body irradiation), or the New York (hyperfractionated total body irradiation and cyclophosphamide conditioning regimen). The majority of patients will not have an HLA compatible donor and will, therefore, proceed to a maintenance chemotherapy regimen. This regimen will include a cyclic sequential program to include modified Capizzi II therapy, followed by four courses of Capizzi I. The modified Capizzi will include two doses of high-dose Ara-C, L-asparaginase and either daunomycin or idarubicin, depending upon the initial randomization. Repeated cycles of maintenance therapy will be given for a total of 2 1/2 years.

CCG-2891 — TREATMENT OF CHILDREN <21-YEARS OF AGE WITH NEWLY DIAGNOSED ACUTE NON-LYMPHOCYTIC LEUKEMIA (ANLL) AND MYELODYSPLASTIC SYNDROME (MDS)

There are two major questions being asked in CCG-2891. These are:

1) Can one improve both remission induction, as well as longterm survival by intensifying induction drugs, especially timing of chemotherapy given without untoward toxicity?
2) Can similar results found after allogenic bone marrow transplantation be obtained using autologous bone marrow rescue with potentially less toxicity, as suggested by European investigators?

CCG-N891 — PARENTS' PERCEPTIONS OF RANDOMIZATION

This is a study to determine factors that influences parents' refusal or agreement to consent to randomization and cooperative group therapeutic studies. Specifically, the goals of this study are:

a) To determine whether parents' knowledge of randomization influences their decision to accept or refuse participation in clinical trials.

b) To assess whether selected demographic factors influence parents' decisions.

c) To explore the possible influence of clinical characteristics, (e.g., diagnosis, prognosis, child's age), on parents' decisions.

d) To determine if parents' perceptions of randomization influence their decisions.

e) To compare early vs. late randomization with respect to the above factors.

The study employs a comparative design using a brief self-administered questionnaire completed by eligible parents.

CCG-E08 — ETIOLOGIC STUDY OF NON-HODGKIN'S LYMPHOMA IN CHILDHOOD

The design of the study is relatively straightforward. When non-Hodgkin's lymphoma is suspect, a sample of the initial tumor tissue will be prepared and sent for the above studies. Blood for viral serology will also be obtained at diagnosis. Within the following weeks to months a very detailed telephone questionnaire

will be given to parents using case-control methodology.

CCG E12 — EPIDEMIOLOGY OF BRAIN TUMORS IN YOUNG CHILDREN

Preconception exposure to various carcinogens and mutagens may play a role in the development of brain tumors. Certain occupational exposures of parents increase the risk of brain cancer in their children. Such associations include parental exposure to pain solvents and other chemicals and metals. Gestational exposures have also been reported to play a role in the development of brain tumors, particularly in nitroso compounds and ionizing radiation. Neurally active drugs may also play a role.

The above list of potential mutagens and carcinogens is vague, incompletely and largely unproven. In order to enact a program to potentially decrease the instance of neoplasms of the brain, more specific data on cause-effect relationships are required. The proposed epidemiologic study has as its goal a definition of the major environmental etiologic factors which play a role in the development of tumors of the brain.

CCG-E13 — CASE CONTROL STUDY OF HODGKIN'S DISEASE IN CHILDHOOD

This is a case control interview study of children with Hodgkin's disease to evaluate previously hypothesized risk factors and to generate new ones. On the basis of epidemiological findings, Hodgkin's disease appears to be three diseases - a childhood disease (affecting those in the age group 0-14 years), a young adult disease (15-44 years), and an older adult disease (usually 50+ years). This study focuses on the childhood form of the disease and will attempt to: a) test a major hypothesis that children with Hodgkin's disease have different patterns of infectious disease than do matched controls; b) evaluate parental occupational exposures as risk factors for childhood Hodgkin's disease; c) evaluate environmental exposures to wood and chemicals as possible risk factors in children; d) evaluate familial aggregation of Hodgkin's disease; e) evaluate risk factors for childhood Hodgkin's disease separately for histological subtypes of the disease and by disease stage and age at diagnosis; and, f) collect serosamples for use in future biological studies.

CCG-E15 — CASE CONTROL STUDY OF ACUTE LYMPHOBLASTIC LEUKEMIA

Acute lymphoblastic leukemia (ALL) is the most common form of malignancy in children under 15 years of age, and the incidence of leukemia appears to be increasing. The present study is designed to examine the role of risk factors for childhood ALL within biologically defined subgroups (i.e., immunophenotyping, morphologic classifications, and karyotypic analysis of diagnostic bone marrow samples). This study will assess whether biologically or clinically distinct subgroups of ALL are also etiologically distinct.

CCG0892 — A PHASE II TRIAL OF HUMAN RECOMBINANT IL-2 GIVEN AS A FOUR-DAY CONTINOUS INFUSION FOR CHILDREN WITH REFRACTORY NEUROBLASTOMA, NON-HODGKIN'S LYMPHOMA, OR SARCOMAS

This is a phase II study designed to determine the anti-tumor effect of IL-2, a single agent therapy for pediatric oncology patients. Because of the IL-2-induced responses observed for adults with melanoma, renal cell carcinoma and lymphoma, it is possible that pediatric

neuroblastoma and lymphoma may potentially have similar susceptibility to this immunologic approach. In vitro data have documented the susceptibility of neuroblastoma cell lines and non-Hodgkin's lymphoma cells to in vitro destruction by IL-2 activated peripheral blood lymphocytes. Separate studies show the susceptibility of pediatric sarcoma cell lines to in vitro lymphocyte-activated killer cell mediated destruction. This trial will therefore focus on neuroblastoma, lymphoma and sarcoma patients.

CCG-0896 — A PHASE II STUDY OF THIOTEPA IN CHILDREN WITH RECURRENT SOLID TUMOR MALIGNANCIES

In view of the recent completion of the Phase I evaluation of Thiotepa in children which shows that the maximum tolerable dose is 65 mg/m², a much higher dose than previously believed, and evidence that rhabdomyosarcoma may be sensitive to Thiotepa, the present Phase II trial of Thiotepa in children with small cell sarcomas (rhabdomyosarcoma, peripheral primative neuroectodermal tumors, Ewing's sarcoma, and undifferentiated small cell sarcomas) and with large cell sarcomas (osteosarcoma and spindle-cell sarcoma) is being undertaken. Patients under 21 years of age when originally diagnosed, who still have tumor present after one or two previous chemotherapy regimens, are eligible for this study provided that they have recovered from the toxic effects of previous chemotherapy, immunotherapy, or radiation therapy, have a peripheral absolute neutrophil count of >1000/mm³, platelet count of >100,000/mm³, have adequate liver function (bilirubin <1.5 x normal, SGRT or SGBT <2.5 x normal), and renal serum function (creatinine <1.5 x normal or creatinine clearance or radioisotope GFR >40ml/min/m²).

Therapy will be Thiotepa, 65mg/m² administered as a five minute intra-venous bolus dose every 3 weeks (21 days) or as soon therafter as haematologic recovery occurs (defined as absolute neutrophil count >1000, platelets >100,000/mm.³) and chemical parameters necessary for patient entry have occurred. Because of the rare occurence of nausea and vomiting and the lack of cystitis or other systemic complications with the use of Thiotepa as described in this protocol, therapy to the child with cancer can be given as an outpatient without the need for pre-hydration. There will be no drug dose escalation. Patients who demonstrate objective evidence of response to Thiotepa in the face of grade III or IV haematologic toxicity or who have treatment delays beyond day 35 from their previous course of treatment will have their subsequent dose of Thiotepa decreased to 50 mg/m². Dose reductions below 50 mg/m² will be done only with the approval of the study chairperson. Therapy will continue every 21 days for 1 year or until disease progression, whichever occurs first.

CCG-L891 — RETROSPECTIVE COHORT STUDY OF LATE EFFECTS IN LONG TERM SURVIVORS OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

The study population will include all patients entered on front line CCSG Studies who are at least 18 years old and who have been incontinuous complete remission. (There are approximately 1400 eligible patients.) Survivors will be traced and an introductory letter will be sent to each patient with address correction requested in the mailing envelope. The complete list with address corrections will be sent to the telephone center for contact or for further tracing where necessary. This will be followed by a u

ephone interview which will average approximately 45 minutes. The relatively few respondents who have a child with a birth defect will be asked a series of questions relating to possible confounding variables which may take an additional 20 minutes. The interview is to cover demographic questions and focus on respondents' health, fertility, the health of their children, risk taking behavior, self-image and depression. In the instances of second cancers and for children with birth defects, permission to seek medical records will be requested, but only in such instances. Survivors will be asked to provide the names of siblings along with the sex and date of birth to provide a pool to be used for controls. If available, one sibling will be selected as a control. Preference will be given to the sibling closest in age. After the data are collected and coded they will be analyzed by single/multiple contingency table methods, with the rate ratio as the measure of association. A further analysis, to evaluate further interactions and controls for confounding, will be done with proportional hazards modules.

PUBLICATIONS

Carter, J.W., Gerrard, J.M., Ready, A.E., Singhroy, S., and **Puta, E.** The effect of excercise on bleeding time and local production of prostacyclin and thromboxane. European Journal of Applied Physiology, **59:**355-359, 1989.

Gerrard, J.M. Measurements of 6-keto prostaglandin $F_{1\alpha}$ and thromboxane B_2 in bleeding time blood: relation to bleeding and vascular disorders. Can. J. Physiol. Pharmacol. **67:**922-928, 1989.

Gerrard, J.M., Taback, S., Singhroy, S., Docherty, J.L., Kostolansky, I., McNicol, A., Kobrinsky, N.L., McKenzie, J.K., and **Rowe, R.** In vivo measurement of thromboxane B_2 and 6-keto-prostaglandin $F_{1\alpha}$ in humans in response to a standardized vascular injury and the influence of aspirin. Circulation **79:**29-38, 1989.

Gerrard, J.M., Docherty, J.C., Israels, S.J., Cheang, M.S., Bishop, A.J., Kobrinsky, N.L., Schroeder, M.L., and **Israels, E.D.** A reassessment of the bleeding time: Association of age, hematocrit, platelet function, von Willebrand factor and bleeding time thromboxane B_2 with the length of the bleeding time. Clin. Invest. Med., **12:**165-171, 1989.

Hornstra, G., VanHouwelingen, A.C., Simonis, M., and **Gerrard, J.M.** Fatty acid composition of umbilical arters and veins: Possible implications for the fetal — EFA status. Lipids **24:**511-517, 1989.

McDonald, B.E., Gerrard, J.M., Bruce, V.N., and **Corner, E.** Comparison of the effect of canola oil and sunflower oil on plasma lipids and lipoproteins and on in vivo thromboxane A2 and prostacyclin production in healthy young men. Amer. J. Clin. Nutr. **50:**1382-1388, 1989.

Pizzi, N.J., Kapoor, S., and **Gerrard, J.M.** Haematology expert system (HES) for tonsillectomy/adenoidectomy patients. SPIE Vol. 1095 Applications of Artificial Intelligence VII (1989) pp 884 - 893.

PUBLISHED ABSTRACTS

Gerrard, J.M., Lint, D., Israels, S.J., Sims, P.J., Wiedmer, T., and **Pugsie, D.** Identification of a 40 Kd dense granule membrane protein which is deficient in a patient with the Hermansky-Pudlak Syndrome. Blood 74:31a, 1989.

Israels, S.J. and **Daniels, M.** Neonatal platelet function: Evaluation of dense granule secretion. Pediatr. Res. **25:**152A, 1989.

Israels, S.J., and **Kobrinsky, N.L.** Serious reactions to desmopressin in a child with cyanotic heart disease. N. Engl. J. Med. 320:1563, 1989 (L).